ENDOCRINE DISRUPTING CHEMICALS AND SENSITIVE REPRODUCTIVE ENDPOINT MEASURES: EVIDENCE FROM THE MIREC AND MIREC-ID COHORT STUDY IN CANADA

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ABSTRACT

Objective: Prenatal exposure to potential endocrine disrupting chemicals may be linked to adverse reproductive health outcomes; however the evidence available in human populations is scarce and inconsistent. The aim of this study was to examine any potential associations between prenatal exposure to select phthalate metabolites, bisphenol A and triclosan and the anogenital distance at birth and the 2D:4D digit ratio at 6-months of age, in male and female infants.

Methods: Approximately 2000 women in their 1st trimester of pregnancy were recruited from across Canada as part of the MIREC Research Platform. Single spot urine samples were collected and analyzed for various environmental chemicals. Of this sample, 525 women were recruited into the follow-up study, MIREC-ID, which measured the anogenital distance and 2D:4D digit ratio in infants. Analyses included descriptive statistics of the chemicals and the sample population, and the fitting of multiple linear regression models.

Results: In female infants, the phthalate metabolite MBzP was negatively associated with the anus-clitoris distance (p=0.002) and positively associated with the 2D:4D digit ratio (p=0.024). The metabolite MEP was positively associated with the anus-clitoris distance (p=0.008). In male infants, the metabolite MEHHP and total BPA were negatively associated with the 2D:4D digit ratio (p=0.021 and p=0.002, respectively). There we no other significant associations observed among the 96 comparisons tested.

Conclusion: In contrast to some other studies, although a few significant associations were observed, in this study, type 1 error cannot be ruled out due to the many comparisons made.

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THESIS ADVISORY COMMITTEE

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1: INTRODUCTION TO THE STUDY

1.1 The Problem

Endocrine disrupting chemicals (EDCs) or 'endocrine disruptors' as they are commonly known, are exogenous chemicals that interfere with the synthesis, storage, release, transport, binding and degradation of hormones (i.e. hormone signalling) (1,2). They can consist of natural substances, or pesticides, plasticizers and metals, many of which are found in personal care products, household items and food packaging (3). These chemicals may have adverse effects on homeostasis, growth, development, metabolism, or neurological or reproductive health (2,4). They are most harmful when exposure occurs during critical periods of development; that is, prenatally or during early postnatal development (5). The presence of EDCs in a range of common products and the tendency for some to persist in nature (6) make them ubiquitous in today's environment, potentially posing a health risk for humans.

Metabolites of many of these chemicals can have estrogenic and/or anti-androgenic properties (3), thereby interfering with the binding of gonadal hormones and potentially inhibiting their biological purpose. Further, the development of the reproductive tract and its comprising organs is highly dependent on the function of these hormones during fetal growth. Currently, of interest in many reproductive toxicity studies and in the media are the possible health effects from exposure to the potential endocrine disruptors: bisphenol A (BPA), phthalates and triclosan (TCS). These health effects may include sexual development of the infant, as measured by anogenital distance (AGD) and the ratio between the lengths of the 2^{nd} and 4^{th} fingers digits (2D:4D digit ratio).

Presently, the evidence around the potential reproductive toxicity and adverse effects of endocrine disruptors is largely based on animal models and needs to be assessed in human populations. Of the few epidemiologic studies conducted, the results are not always consistent and thus must be validated with additional research. Moreover, there is a paucity of data on the reproductive toxicity of these chemicals in a Canadian population. As such, this thesis will be the first to examine the possible reproductive risks of exposure to these priority environmental chemicals in a population of Canadian infants.

1.2 Purpose Statement

This thesis project will entail the analysis and interpretation of data collected from a large prospective cohort study conducted in Canada that had as a primary objective to examine prenatal and lactational exposure to environmental chemicals and the possible effects on maternal and infant health. The purpose of this thesis project is to model the association between maternal urinary levels of several priority environmental chemicals and markers of reproductive health in Canadian newborns, while controlling for potential confounding variables.

1.3 Objectives

The objectives of this thesis project are:

Objective 1. To examine potential associations between prenatal exposure to BPA, TCS or select phthalate metabolites and the anogenital distance in newborn male and female infants.

Objective 1a. To examine potential association between prenatal combined exposure to various phthalate metabolites, by molecular weight and anogenital distance in newborn male and female infants.

Objective 2. To examine potential associations between prenatal exposure to BPA, TCS or select phthalate metabolites and the ratio of the lengths of the 2nd and 4th fingers digits in male and female infants at 6-months of age.

Objective 2a. To examine potential association between prenatal combined exposure to various phthalate metabolites, by molecular weight and the ratio of the lengths of the 2nd and 4th fingers digits in newborn male and female infants, at 6-months of age.

Objective 3. To examine the correlation between the anogenital distance and the ratio of the lengths of the 2nd and 4th fingers digits.

1.4 Hypotheses

It is hypothesized that:

Hypothesis 1. Higher levels of maternal exposure to BPA, TCS or select phthalate metabolites will be associated with a shortened anogenital distance.

Hypothesis 2. Higher levels of maternal exposure to BPA, TCS or select phthalate metabolites will be associated with a larger ratio of the lengths of the 2nd and 4th finger digits.

Hypothesis 3. There will be a statistically significant correlation between the anogenital distance and the ratio of the lengths of 2nd and 4th finger digit ratios.

1.5 The Public Health Significance

This thesis will add to the growing evidence surrounding the potential harm associated with exposure to endocrine-disrupting chemicals in today's environment amongst susceptible populations, such as pregnant women and their infants. Specifically, this study will examine how early life exposure to these chemicals may affect key endocrine sensitive endpoints. This information can guide future studies that employ a longitudinal study design looking at how these endpoints change throughout childhood and adult years, and suggest how development at birth may be an indicator of adverse conditions later in life. Moreover, results from this thesis project may help strengthen health risk assessments, contributing to the improvement of public health, consumer products, food safety and the environment.

CHAPTER 2: REVIEW OF THE LITERATURE

This literature review provides a brief introduction to endocrine disruption, including speculation into possible endocrine disruptors: bisphenol A, select phthalate metabolites and triclosan. It also presents evidence surrounding the possible reproductive toxic effects of these chemicals, specifically related to the development of the anogenital distance and the 2D:4D finger digit ratio. Further, a brief summary of biomonitoring is also presented.

2.1 Endocrine Disrupting Chemicals

Hormones are 'chemical messengers' that are produced by various endocrine glands in the body, including the pituitary gland, pineal gland, thymus, thyroid, adrenal gland, testes (in males) and ovaries (in females). They travel through the bloodstream to cells that make up the various tissues and organs, binding to receptors on cell membranes, in the cytoplasm and the nucleus of cells; this initiates gene activity and a variety of physiological processes (7).

As defined by the United States Environmental Protection Agency (USEPA), an endocrine disrupting chemical is an 'agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior' (1–3). There are a vast number of chemicals that can be classified as potential endocrine disruptors, including both natural and synthetic chemicals. Natural substances include a group of compounds called phytoestrogens – which are plant-derived estrogens that are found in many food sources, including soy products (3). Further, EDCs can be found in: pesticides (e.g. dichlorodiphenyl-trichloroethane); fungicides (e.g. vinclozolin); substances used in the

creation of plastics or as plasticizers (e.g. BPA, phthalates); industrial solvents/lubricants and their by-products (e.g. polychlorinated biphenyls, dioxins); metallic items (e.g. cadmium, mercury, lead, uranium, arsenic, manganese, zinc); and pharmaceutical agents (e.g. diethylstilbestrol) (3,7). Many of these synthetic chemicals are found in personal care products, cosmetics, household items, cleaning products and food packaging (3).

Endocrine disruptors interfere with hormone-signalling processes involved in growth, development, metabolism, reproduction and behaviour (1,2). Although the mechanism of EDC action is very diverse, there are several key ways they can act. These include: binding to the receptor and adding to normal hormonal signalling; binding to the receptor and blocking normal hormonal signalling; interfering with natural hormone synthesis (increasing or decreasing the availability of the hormone); interfering with hormone metabolism or hormone transport and storage (also increasing or decreasing the availability of the hormone); and lastly, affecting the total levels of a mature hormone receptor via disruption or modulation of gene expression, folding, or transport (7).

2.2 Endocrine-sensitive endpoints

Measurement of endocrine sensitive endpoints within the first years of life is a tool used to determine if there may have been any health effects from prenatal exposure to endocrine disrupting chemicals (8). Among other measures, anogenital distance (AGD) is a sensitive marker of fetal androgen/anti-androgen exposure frequently used in animal studies (9,10) and more recently, human studies (11–15). AGD is the distance between the genitals and the anus in both boys and girls (8). Several studies have shown the sexually dimorphic nature of AGD in humans (16,17). Males have an AGD that is 2-2.5 times greater than

females, which is evident from birth up to 24-30 months; a smaller difference exists in childhood and adult years (12,14,18,19). The male-female difference in AGD can likely be attributed to fetal androgen exposure as studies have shown the absence of the sexual dimorphism nature of the AGD in mice with androgen insensitivity (20). In addition to the AGD, several studies have looked at the anoscrotal distance (ASD) and the anogenital index (AGI) (see Table 1). The ASD is the distance between the anus and scrotum in males, while the AGI is a weight normalized measure of the AGD (i.e. AGD/weight of infant).

There have been a small number of studies that suggest that alterations to the AGD as seen at birth may be associated with later reproductive health outcomes. In males, a shorter AGD has been shown to predict poorer semen quality (21), reduced testosterone and testicular volume (22) and potentially a higher probability of infertility (23). In females, a larger AGD has been associated with congenital adrenal hyperplasia (16) and multifollicular ovaries (24).

Additionally, several studies suggest that the ratio between the length of the second and fourth fingers (2D:4D finger digit ratio) may also be a useful measure of fetal androgen or anti-androgen exposure, as observed in rodents (25–29). On average, males have a longer fourth finger relative to their second finger compared to females at birth (i.e. a smaller 2D:4D digit ratio) (30–32). This sexual dimorphism has been thought to reflect differential fetal androgen exposure, as digit length and digit length ratio have been shown to be correlated with prenatal testosterone and estrogen (33).

Alterations to the 2D:4D digit ratio as seen at birth may be associated with adverse health outcomes in later years of life. One study found that low 2D:4D digit ratio was related to a higher body mass during childhood and adolescence, higher body mass index, a higher

number of children among fathers and higher testosterone levels during adulthood (34). However, it is difficult to attribute any adverse health outcomes as being solely the result of alterations to the 2D:4D digit ratio seen at birth as the ratio increases with age in both sexes and is highly dependent on postnatal and pubertal factors (26).

2.3 EDCs of interest

Experimental studies with animals have shown that exposure to elevated concentrations of EDCs during critical periods of development may be associated with reproductive toxicity. Two important groups of endocrine disruptors that have been studied widely are phthalates and BPA (35). One that has been less studied, but is of interest due to its widespread use is TCS.

2.3.1 Phthalate metabolites

Phthalates or 'phthalate esters' as they are also known, are a group of industrial chemicals that are added to plastics to increase their flexibility and durability (36–38). Phthalates can be found in a vast number of consumer products. High molecular weight phthalates such as di(2-ethylhexyl) phthalate (DEHP) are primarily used as plasticizers and are found in automotive plastics, detergents, lubricating oils, vinyl tiles and flooring, pharmaceuticals, medical devices and toys. Alternatively, low-molecular phthalates such as diethyl phthalate are found primarily in personal-care products, including soap, shampoo, deodorants, lotion, fragrances, hair products and nail polish (39). Exposure to phthalates is widespread, mainly due to their relatively easy ability to enter the environment. Populations are exposed to phthalates primarily through diet, but also through inhalation and dermal

contact (38,40,41). Upon absorption, phthalates are metabolized as mono- or oxidized phthalate metabolites; they are then excreted in urine and feces (42). For example, the parent phthalate DEHP is first metabolized to mono-(2-ethylhexyl) phthalate (MEHP), and then further metabolized to mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP) (40). In Canada, several phthalate metabolites have been detected in more than 95% of the population, as determined from the results of the Canadian Health Measures Survey (CHMS) (43).

Several phthalate metabolites have estrogenic/anti-androgenic properties. Rodents exposed *in utero* to several phthalate metabolites during the first trimester of pregnancy – said to be the critical period of development of the reproductive tract (44) – show a disruption in their androgen-signalling pathway (45–48). This results in an inhibition of the synthesis of fetal testosterone (via leydig cells), potentially leading to multiple male reproductive abnormalities. For example, early exposure to the phthalate metabolites of dibutyl phthalate (DBP), DEHP and benzyl butyl phthalate (BBzP) have been shown to be associated with a syndrome of genital dysmorphologies in male rats. These dysmorphologies include shortened AGD, hypospadias (abnormality of the urethra and penis), cryptorchidism (absence of one or both testes from the scrotum), incomplete testicular descent, smaller testis weight, smaller penile size and malformations of the epididymis, vas deferens, seminal vesicles and prostate organs (47). This has often been collectively known as the ‘phthalate syndrome’ (47). Human studies suggest a similar effect of phthalate exposure. In the study conducted by Swan (15), it was found that there was an increased risk of reduced penile size with exposure to the phthalate metabolite MEHP (a DEHP metabolite), and an increased risk of incomplete testicular descent associated with exposure to the DEHP phthalate

metabolites MEHP, MEHHP and MEOHP. Though there have been several studies that have looked at the association between maternal phthalate exposure during pregnancy and adverse outcomes at birth, more research is needed to validate and confirm these findings.

2.3.2 Bisphenol A (BPA)

Bisphenol A is an industrial chemical used widely in the production of polycarbonate plastics and epoxy resins, which are both commonly found in a number of consumer products. Polycarbonate plastic, a form of hard, clear plastic, is used in the production of food and drink packaging, including beverage bottles and food containers (49). They can also be found in compact discs, automobile parts, plastic dinnerware, eyeglass lenses, toys and a variety of safety equipment. On the other hand, epoxy resins, which act as a protective lining on the inside of metal-based products, can be typically found in canned foods, bottle tops, resin-based paints, flooring, thermal paper, dental sealants and other medical devices (42,50–53). Due to its lipophilic nature, BPA enters the environment mainly through product leaching, especially when the product is heated, washed or put in any sort of stress (54). The main sources of exposure to BPA include diet, but can also include water and air, to a minimal degree (55,56). Studies have shown that increased ingestion of foods that are packaged in BPA-containing plastics and metals have resulted in a rapid increase in BPA levels in urine, blood and other biospecimens. In a national survey conducted in Canada, 91% of the population had detectable concentrations of BPA in their urine at a given time (57).

Due to its wide availability and presence in the environment, the potential adverse effects of BPA exposure have been studied extensively. Elevated maternal urinary BPA

concentrations have been shown to be associated with pre-term birth (58); shortened gestational length (59); lower growth rates and a smaller head circumference (51). Further, there have been several studies that have looked at the association of elevated BPA exposure and birth weight, reporting conflicting results. While some studies suggest that increased maternal BPA levels are associated with low birth weight (60,61), others report no such association (62,63). In animal studies, the effects of BPA on offspring has been shown to be dose dependent, where low dose BPA exposure was associated with a lower weight while high dose exposure was associated with an increased weight (51). Other studies conducted in animals have suggested that maternal BPA exposure may impact the proper delivery of offspring (64). Additionally, several studies with rodents have shown that *in utero* exposure to low environmental levels of BPA feminizes 2D:4D finger digit ratio in males by a similar mechanism of modifying fetal testosterone levels (32). This suggests that exposure to anti-androgenic agents such as BPA during the critical period of genital tract development has the potential to de-masculinize male offspring.

2.3.3 Triclosan (TCS)

Another chemical that is becoming more widely recognized as an endocrine disrupting chemical is triclosan (65,66), mainly due to its similarity in structure to known EDCs and limited evidence from experimental studies of effects on hormones (67,68). TCS is an anti-microbial agent, commonly found in personal care products, household items and medical devices (66). In Canada, TCS was detectable in 75% of the total urine samples collected as part of the 2009-2011 Canadian Health Measures Survey (43). To date, only two studies have looked at prenatal TCS exposure, both of which reported no adverse health

effects on birth weight, length or head circumference (63,69). One study that analyzed urine samples from 2003-2008 NHANES survey of the U.S. population, found a positive association between TCS exposure and body mass index (70). A study by Velez et al. (68) found that elevated TCS exposure may be associated with diminished fecundity. Though there have been no studies that have looked at TCS and the AGD or 2D:4D digit ratio, TCS exposure has been shown to decrease the concentration of testosterone in male rats in a dose-dependent manner (71). Further, several studies have shown that TCS may possess both androgenic and estrogenic-like properties, though there seems to be some disagreement amongst the information presented in literature (72).

2.3.4 Summary of studies

There have been a relatively small number of studies conducted that directly compare *in utero* exposure to potential endocrine disrupting chemicals and sensitive reproductive endpoint measures in a human population. A comprehensive literature review was performed in PubMed to identify any articles that have looked at the association between *in utero* exposure to BPA, TCS and phthalate metabolites and AGD or 2D:4D digit ratio in human populations. The search was based on keywords related to BPA, TCS and phthalate metabolites and AGD and 2D:4D digit ratio. There were no language or time restrictions in the search strategy. Only studies conducted in human populations were included in the final selected articles.

No studies were identified that have looked at the association between *in utero* exposure to TCS and anogenital distance. Further, no studies have looked at the association between *in utero* exposure to BPA, TCS or phthalate metabolites and the 2D:4D digit ratio.

TABLE 1: ASSOCIATION BETWEEN *IN UTERO* EXPOSURE TO BPA, TCS OR PHTHALATE METABOLITES AND ANOGENITAL DISTANCE IN HUMAN POPULATIONS

Author/Year	Population	Exposure	Key Findings	Key Non-Significant Findings
Phthalate Metabolites				
Bornehag et al., 2014 (73)	Swedish male infants (n=196) at 21 months of age.	MBP, MBzP, MEP, and metabolites of parent phthalate compounds DEHP and DiNP; measured from first-trimester maternal urine.	Inverse association between exposure to 2 DiNP phthalate metabolites, and sum of metabolites and ASD (p=0.029-0.047).	No significant association found between exposure to MBP, MBzP, MEP, DEHP metabolites and ASD or AGD in male infants.
Bustamante-Montes et al., 2013 (74)	Pregnant females $\geq$ 18 years of age in Toluca, Mexico (n=174). Singleton pregnancy with no serious health conditions. Only females who gave birth to male infants were included (n=73).	MBP, MBzP, MEHP and MEP measured from third-trimester maternal urine.	Inverse association between total phthalate exposure (MBP, MBzP, MEHP, and MEP) and AGD (p=0.037), penile width (p=0.050) and penis stretch length (p=0.034).	No significant association between exposure to single phthalate metabolites MBP, MBzP, MEHP, and MEP and AGD in male infants.
Huang et al., 2009 (75)	Pregnant women who planned to undergo amniocentesis (n=65). Male and female infants included.	MBP, MBzP, MEHP, MEP, and MMP measured from maternal urine and/or amniotic fluid prior to amniocentesis.	Significant negative correlation between amniotic fluid MBP and AGD (p<0.06) and AGI adjusted by birth weight (p<0.05) in female infants.	No significant correlations between exposure to MBP, MBzP, MEHP, MEP, and MMP and AGD/AGI in male infants.
Swan et al., 2005 (14)	Women from the Study	MBP, MBzP, MCPP,	Urinary concentrations	No significant associations

	for Future Families multicenter pregnancy cohort study in the US (≥ 18 years of age) (n=85, male infants only).	MEP, MiBP, MMP and metabolites of parent phthalate DEHP measured from maternal urine late in pregnancy (mean=28.3 weeks).	of MBP, MBzP, MEP, and MiBP inversely related to AGI (p=0.007-0.097). Combined phthalate exposure also inversely related to AGI (p=0.0009).	between exposure to DEHP metabolites (MEHHP, MEHP, and MEOHP), MCPP or MMP and AGI.
Suzuki et al., 2011 (13)	Pregnant females in Tokyo, Japan (n=111). Gave birth to male newborns.	MBzP, MEHHP, MEHP, MEOHP, MEP, MMP, and MnBP measured from maternal urine samples obtained from to the 9^{th} to 40^{th} week of gestation (mean=29 weeks).	Urinary concentration of MEHP negatively correlated to AGI (p=0.047). Maternal smoking status was positively significant in predicting the AGI (p=0.011).	No significant associations between exposure to combined DEHP metabolites (MEHHP, MEHP, MEOHP), MBzP, MEHHP, MEOHP MEP, MMP, and MnBP, and AGI.
Swan et al., 2015 (76)	Women from the Infant Development and the Environment Study (TIDES) (n=753). Less than 13 weeks pregnant.	MBzP, MCNP, MCOP, MCPP, MEP, MiBP, MnBP, and metabolites of parent compound DEHP measured from first trimester maternal urine samples.	Urinary concentrations of DEHP metabolites MEHHP, MEHP, and MEOHP inversely associated with AGD and ASD in male infants (p=0.036, 0.008 and 0.013, respectively). Sum of DEHP also related (p=0.029).	No significant association between other phthalate metabolites and AGD in male infants. No associations found in female infants.
Bisphenol A				
Miao et al., 2011 (77)	Retrospective cohort study in boys with parents exposed to BPA	Parental BPA exposure during index pregnancy estimated based on the	Parental occupational exposure to BPA during pregnancy associated	None.

	in China (n=153).	combination of the current exposure level (measured using air sample monitoring) and other lifestyle factors. Urine samples were also collected for a sample of participants to verify the validity of classification of BPA exposure.	with shortened AGD in male offspring ($p<0.01$). Also dose-response relationship with increased BPA exposure levels in pregnancy associated with a greater magnitude of shortened AGD ($p=0.008$).	

AGD=anogenital distance; AGI=anogenital index; ASD=anoscrotal distance; BPA=bisphenol A; DEHP=Di(2-ethylhexyl) phthalate; DiNP=Diisononyl phthalate; M(n)BP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCNP=mono-(3-carboxypropyl) phthalate; MCOP=Mono(carboxyoctyl) phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate; MiBP=Mono-isobutyl phthalate; MMP=Mono-methyl phthalate.

The findings from these studies summarized in Table 1 yield interesting results, however, in many cases are not entirely consistent with each other. In total, 6 studies have looked at the association between *in utero* exposure to various phthalate metabolites and AGD (including both the distance to the penis or scrotum) or the AGI in a human population. Only two studies conducted have included female infants, in addition to male infants (75,76). The findings by Swan et al. (14) suggest that the phthalate metabolites MBP, MBzP, MEP and MiBP are inversely related to the AGI in male infants. Further, the study conducted by Huang et al. (75) found the phthalate metabolite MBP to be inversely related to the AGD and AGI (in female infants only). However, the results of the other studies found no such association between these metabolites and the AGD in both male and female infants (13,73–76). In regards to phthalate metabolites from the parent compound DEHP, Swan et al. (76) found MEHHP, MEHP and MEHOP to be inversely related to the AGD and ASD in male infants, in addition to the sum of these metabolites. The study by Suzuki et al. (13) also found the phthalate metabolite MEHP to be significantly associated with the AGI in male infants, but found no similar association with other DEHP phthalate metabolites or the sum of metabolites. Similarly, no such association was found in other studies (14,73–75). No other associations were found between phthalate metabolites and the AGD in female or male infants.

There has been only one study (retrospective) that has looked at *in utero* exposure to BPA and AGD in humans. This study found that parental occupational exposure to BPA during pregnancy was associated with shortened AGD in male offspring, in addition to a dose-response relationship with increased BPA exposure levels in pregnancy associated with a greater magnitude of shortened AGD (77).

It is important to note that many of these studies had several limitations. This included the use of single spot urine samples, which may have led to exposure misclassification, timing of exposure measurement, small sample sizes and the presence of potential residual confounding.

2.4 Biomonitoring

Biomonitoring is the "measurement of a chemical, the products it makes after it has broken down, or the products that might result from interactions in the body" (78); it is a tool used to assess exposure to environmental chemicals in human biological specimens. These specimens typically include blood and urine, but can also include breast milk, amniotic fluid, meconium, and seminal fluid (79). Biomarkers, which provide the best measure of the internal dose of a chemical from any route of exposure, have several categories. These include: biomarkers of exposure, biomarkers of susceptibility and biomarkers of effect (80). A biomarker of exposure is the chemical itself, including its metabolites, reaction products and/or any endogenous surrogates. Biomarkers of susceptibility are factors that make an individual (in the case of humans) more susceptible to the effects of chemical exposure; this can include genetic factors and other biological factors, such as: lifestyle, nutritional status etc. Biomarkers of effect are indicators of change in biologic function – these can be biochemical, physiological or behavioural – which are related to exposure to an environmental chemical and are typically associated with observed adverse health effects in their early stages.

2.4.1 Advantages and Disadvantages

Over the years, biomonitoring has become the gold standard to measure environmental exposure to numerous contaminants that may be toxic. Biomonitoring can provide an accurate representation of the concentration of active chemical in an individual's body; it measures integrated exposure from all routes and sources of exposure in addition to being able to detect relatively low levels of exposure (81). Further, from a public health and policy level, biomonitoring has several advantages, including establishing baseline levels of a chemical in a specified population, comparing exposure to environmental chemicals amongst different populations, supporting risk management efforts and providing data for other research needs (78). In Canada, biomonitoring data is readily collected through the Canadian Health Measures Survey, which is an ongoing survey that collects information from Canadians about their health, and includes measurement of various chemicals in blood and urine (78). Results from cycle 2 (years 2009-2011) present updated Canadian biomonitoring data on exposure to environmental chemicals (43).

Biomonitoring has several key limitations as well; this includes its inability to define sources, pathways and duration of exposure, and to define toxic dose levels, making it difficult to interpret. In addition, biomonitoring cannot identify meaningful reference levels of many chemicals – in part, due to the presence of 80,000-90,000 manmade chemicals in the world today. Thus, exposure levels of a particular chemical in a population may be at toxic level, but may be considered 'normal' levels. Lastly, it can require numerous resources and can be quite a burden to individuals participating in a biomonitoring study (81).

2.4.2 Urine biomonitoring

One of the most commonly used matrices in human biomonitoring is urine. One of the main advantages with using urine is the ease and convenience of collection, especially when collecting a single sample (spot-urine sample) (82). However, the key issue with the collection of a single sample is that for chemicals that have a short biological half-life, a single spot urine sample may not accurately reflect accumulated exposure to the chemical of interest, potentially leading to exposure misclassification. Thus, it has been suggested that urine should be collected as frequently as possible during a specified risk period (83). This was further reiterated in a study by Fisher et al. (84) that looked at daily and across pregnancy variability in BPA and phthalate metabolite urinary concentrations; the investigators found that the time of day that urine is collected is a significant predictor of exposure levels, suggesting that exposure levels of a particular chemical may be more accurate if multiple urine measurement are collected (84). Though collection of frequent urine samples may more accurately represent exposure to a particular chemical, it may result in incomplete collection as patient compliance may be lower (82), which in turn may lead to selection bias, causing a distortion in the relationship between the exposure and the outcome (85). In addition, there are increased costs especially the laboratory analysis of multiple urine samples per individual.

CHAPTER 3: METHODS

3.1 Study design

Data for this research project were collected from the Maternal-Infant Research on Environmental Chemicals (MIREC) Study and the Maternal-Infant Research on Environmental Chemicals – Infant Development (MIREC-ID) Study, a follow-up study to MIREC. Both of these studies, along with others are part of the larger MIREC Research Platform, a collaboration between scientists at Health Canada, Sainte Justine's Hospital in Montreal, Quebec, and clinical and academic researchers throughout Canada. This research platform was established to support the Chemicals Management Plan (CMP) that was launched in 2006 by the Government of Canada. The CMP is an initiative that allows the government to work closely with health, environment and consumer groups as well as industry to set clear priorities in the assessment and handling of chemicals in order to protect the environment and the health of Canadians (86). The MIREC Research Platform is primarily funded by Health Canada, with contributions from the Ontario Ministry of the Environment and the Canadian Institutes of Health Research (CIHR).

MIREC is a large cohort study that was conducted across Canada in pregnant women and their children in order to assess human exposures and potential health risks from prenatal exposure to various environmental chemicals. The primary objectives of the MIREC study were the following: 1) to determine whether current non-occupational exposure to selected metals is related to elevated maternal blood pressure or fetal growth restriction and 2) to obtain national-level biomonitoring data of *in utero* and lactational

exposure to environmental chemicals that may have adverse effects on human health. Although national data on exposures to environmental chemicals are collected through population-based surveys (e.g. Canadian Health Measures Survey), such assessments may not be successful in collecting extensive data on pregnant women and their infants. Pregnant women are a susceptible and vulnerable population and thus require specialized techniques (85). Following the initiation of the MIREC study, additional funding was secured to allow for follow-up studies, including MIREC-ID. MIREC-ID was established to take advantage of the clinical and laboratory data collected during MIREC, in order to assess the effects of prenatal exposures to environmental chemicals on a range of developmental outcomes at birth and at 6-months of age in a sub-sample of the MIREC infants. The size of the infant cohort was limited by the funds available. Further, the MIREC data and biological specimens bank (named 'MIREC Biobank') was also created to store data and biospecimens collected for future research.

3.2 Study population

The MIREC study recruited pregnant women from the general Canadian obstetrical population. These women were attending prenatal clinics (ultrasound, midwife and/or doctor's clinics) and at the time were in the 1st trimester of pregnancy (6 -14 weeks). The eligibility criteria included being 18 years of age or older, with the ability to consent and communicate in English or French, being less than 14 weeks gestation, planning to deliver at a local hospital and agreeing to participate in the cord blood collection component of the study. Women with known fetal abnormalities or fetal chromosomal or major malformations in their current pregnancy were excluded from the

study, in order to ensure a healthy obstetric population was obtained. Women with a history of several medical conditions were also excluded from the study. These included: renal disease with altered renal function, epilepsy, any collagen disease such as lupus erythromatosus and scleroderma, active and chronic liver disease (hepatitis), heart disease, serious pulmonary disease, cancer, hematologic disorder (patients with anemia or thrombophyllias were included), threatened spontaneous abortion (women with previous bleeding in first trimester could be included if the site documented a viable fetus at the time of recruitment) and illicit drug use.

The MIREC-ID follow-up study recruited 525 women; these women were recruited during a maternal interview for the MIREC study (before delivery). In addition to meeting the MIREC study requirements, to be eligible for the MIREC-ID study, the infant must have been a singleton without any major congenital birth defects or neurological disorders. The singleton inclusion criterion was required for another component of the MIREC-ID Study unrelated to the objective of this analysis.

3.3 Participant recruitment

Recruitment for MIREC & MIREC-ID was done from 2008-2011 in 10 cities across Canada. These included: Vancouver, Edmonton, Winnipeg, Toronto, Hamilton, Sudbury, Kingston, Ottawa, Montreal and Halifax. As these were multi-center studies, the research staff from each site participated in standardized training. This included training in patient screening, recruitment, consenting, specimen and data collection and processing, as well as shipping biospecimens. In addition, examination techniques of the research staff were monitored and checked regularly.

Initially 8,716 women were approached for participation in the MIREC study. Of those, 5,108 women were eligible and 2,001 consented. After 18 women withdrew from the study and asked that all their data be destroyed, 1,983 women completed the first assessment. As the study progressed, some participants partially withdrew and some were lost to follow-up; 1,727 women completed the final delivery assessment of MIREC.

Subsequently, there were 538 mother-infant pairs recruited for the MIREC-ID study from 6 of the 10 sites. Of those, 525 pairs participated after assessment for eligibility and providing consent. Of the 525 pairs, 426 birth visits were completed and 443 6-month visits were completed. These numbers differ as 82 pairs completed the birth visit only, 99 pairs completed the 6-month visit only and 344 pairs completed both the birth and 6-month visits. Thus, not all of the total 525 mother-infant pairs completed both assessments.

Upon merging of the two datasets (MIREC and MIREC-ID), the study population for this research project consisted of 396 mother-infant pairs in the birth population and 418 mother-infant pairs in the 6-month population. It should be noted that upon merging of the two datasets, the overall sample size is much smaller.

3.4 Ethics and informed consent

The MIREC and MIREC-ID study protocols, questionnaires, consent forms and recruitment posters/pamphlets received ethics approval from human subjects' research ethics committees. These included the Research Ethics Board at Health Canada, the research ethics committee at the coordinating center at Ste-Justine's Hospital in Montreal, as well as the academic and hospital ethics committees at each research site across

Canada (>10). Potential participants were provided with the objective and design of the studies and were asked to sign consent forms prior to beginning the study. Consent forms agreeing to participate in the MIREC study as well as for the use of the stored biological specimens for follow-up studies are included in Appendix 1 and Appendix 2, respectively. Additionally, the consent form agreeing to participate in MIREC-ID study is included in Appendix 3. Participants had the option of partially withdrawing (all data and biospecimens retained) or completely withdrawing (all data and biospecimens destroyed) from the study, at any point in the process. No information was collected on the non-participants as research ethics committees denied our request to collect any information on women who refused to participate in this study, and no sampling frame was used to identify potential participants, which would have contained some data on non-participants.

3.5 Data/biospecimen collection

In the MIREC study, during each trimester, at delivery and in the early postnatal period (up to 10 weeks), various biospecimens were collected from participants and stored for laboratory analyses. These included: maternal blood, umbilical cord blood, urine, infant meconium, human milk and postnatal maternal hair. In addition to the collection of biospecimens, prenatal questionnaires were administered during each visit by trained research staff. During the 1st visit (between $6^{0/7}$ and $13^{6/7}$ weeks), information on sociodemographic characteristics, medical history (obstetrical and non-obstetrical), family history and nutritional supplements was obtained. Further, information on various characteristics of the participants was also collected. This included employment status,

environmental exposures, smoking history (active and passive), alcohol consumption, place of residence, daily activities, nutrition /diet and sunlight exposure. Following this visit, the mother was asked to fill out a nutrient supplements questionnaire at home. During the 2nd visit (between $16^{0/7}$ and $21^{6/7}$ weeks), information on gestational age and current medication use was obtained, in addition to the administration of a food frequency questionnaire. Also during this visit, any clinical laboratory tests the mother had completed were obtained from medical chart reviews. During the 3rd visit (between $32^{0/7}$ and $34^{6/7}$ weeks), similar questions on lifestyle as collected during the 1st trimester visit were asked. During all three pre-delivery visits and at delivery, sampling characteristics of the biospecimens collected were also recorded, in addition to blood pressure and anthropometric measurements of the mother. The detailed status of the pregnancy was recorded during all visits as well. For pregnancies greater than 20 weeks, a chart review questionnaire was completed after delivery. This questionnaire assessed: glucose tolerance during pregnancy, corticosteroid use during pregnancy, blood pressure during pregnancy prior to admission for delivery, blood pressure after admission for delivery, anthropometric measurements prior to delivery, maternal conditions after admission and maternal outcomes after delivery. A lactational questionnaire and milk collection sheet was administered 2-10 weeks after delivery. Lastly, information on the labour and delivery of the baby and neonatal characteristics were recorded. In cases where there were multiple pregnancies, neonatal information for all babies was obtained.

MIREC-ID was a follow-up study that consisted of in-clinic assessments conducted at birth and 6 months of age. During the birth/neonatal period (within 24-48 hours after birth), short-term outcomes of growth (i.e. anthropometric measurements) and

sexual development were assessed. Further, sociodemographic characteristics and anthropometric measurements of the father were obtained. Any infant medication and or vitamins taken by the infant were also recorded. At 6 months of age, the follow-up visit included review of the infant birth medical chart and a self-administered questionnaire for the mother. The information obtained from the infant at 6-months of age included the assessment of their sensory development (i.e. auditory and visual testing), sexual development, growth and cardiac functions. All infant testing at birth and 6-months was performed in the presence of the mother. During this visit, a maternal interview was also conducted to collect data on sociodemographic characteristics and environmental exposures, in addition to infant characteristics.

3.6 Exposure assessment

Phthalates, BPA and TCS – along with other environmental chemicals – were measured in maternal urine collected during the first trimester (6-13 weeks) of pregnancy of the MIREC study. A minimum of 80 ml of urine was collected in a 125 ml Nalgene® container (Thermo-Fisher Scientific Inc., Rochester NY, USA), which was assured to be BPA and phthalate-free. Aliquots in similar containers were frozen at -20ºC within 2 hours of collection and were subsequently sent to the MIREC coordinating center at Ste.-Justine's Hospital in Montreal. They were stored at -30ºC prior to being sent for analysis to the Centre de toxicologie du Quebec (CTQ), Institut national de santé publique du Québec (INSPQ). The INSPQ is accredited by the Standards Council of Canada under ISO 17025 and CAN-P-43, which is the international standard for technical competence

and quality in all areas of testing and calibration. Urine samples were analyzed for several environmental chemicals, including those of interest for this thesis project.

When ingested, BPA and TCS are metabolized into their respective conjugate forms by the liver; they are then excreted from the body via urination. For this reason, this thesis project considered the analysis of urinary concentrations of total and free BPA and TCS. 'Total' being the free plus conjugated forms of the chemicals and 'free' being the unconjugated forms of the chemicals, which are presumed to be more biologically active.

Furthermore, when phthalates are absorbed into the body, depending on the parent phthalate compound, various mono- and oxidative phthalate metabolites are formed and excreted in the urine. These are presented in Table 2 below.

TABLE 2: PARENT PHTHALATES AND URINARY METABOLITES

Phthalate Name (Parent Metabolite)	Abbreviation	Urinary Metabolite(s)	Abbreviation	Analyzed in MIREC?
Butyl benzyl phthalate	BBzP	Mono-benzyl phthalate	MBzP	Yes
Di-n-butyl phthalate	DnBP	Mono-n-butyl phthalate	MnBP	Yes
Di-cyclo-hexyl phthalate	DCHP	Mono-cyclo-hexyl phthalate	MCHP	Yes
Diethyl phthalate	DEP	Mono-ethyl phthalate	MEP	Yes
Di-(2-ethylhexyl) phthalate	DEHP	Mono-(2-ethylhexyl) phthalate	MEHP	Yes
		Mono-(2-ethyl-5-hydroxy-hexyl) phthalate	MEHHP	Yes
		Mono-(2-ethyl-5-oxo-hexyl) phthalate	MEOHP	Yes
		Mono-(2-ethyl-	MECPP	No

		5-carboxypentyl) phthalate		
Di-iso-nonyl phthalate	DiNP	Mono-isononyl phthalate	MiNP	Yes
Di-isodeycl phthalate	DiDP	Mono-(carboxynonyl) phthalate	MCNP	No
Dimethyl phthalate	DMP	Mono-methyl phthalate	MMP	Yes
Di-n-octyl phthalate	DnOP	Mono-(3-carboxypropyl) phthalate	MCPP	Yes
		Mono-n-octyl phthalate	MnOP	Yes

Thus, this thesis project considered urinary concentrations of 11 phthalate metabolites: Mono-benzyl phthalate (MBzP; LOD:0.20 μg/L), Mono-*n*-butyl phthalate (MnBP; LOD:0.20 μg/L), Mono-cyclo-hexyl phthalate (MCHP; LOD:0.20 μg/L), Mono-ethyl phthalate (MEP; LOD:0.50 μg/L), Mono-(2-ethylhexyl) phthalate (MEHP; LOD:0.20 μg/L), Mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP; LOD:0.40 μg/L), Mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP; LOD:0.20 μg/L), Mono-isononyl phthalate (MiNP; LOD:0.40 μg/L), Mono-methyl phthalate (MMP; LOD:5.00 μg/L), Mono-(3-carboxypropyl) phthalate (MCPP; LOD:0.20 μg/L), and Mono-n-ocytl phthalate (MnOP; LOD:0.70 μg/L).

For the measurement of urinary BPA and TCS, an enzymatic hydrolysis was used to free the conjugated form of the compound from the urine sample, which was based on a previously developed method (87). The samples were then derivatized at 70ºC (pentafluorobenzylation) for 2 hours followed by extraction with a mixture of hexane and dichloromethane and analyzed by GC Agilent 6890N GC-MS/MS (Agilent Technologies; Mississauga, Ontario, Canada) together with a Quattro Micro GC tandem mass

spectrometer (Waters; Milford, Massachusetts, USA). The measurement of ions generated was performed in multiple reaction-monitoring (MRM) mode with a source in negative chemical ionization mode (NCI). The analytical column used was a HP-5MS 30 m x 0.25 mm i.d. x 0.25 µm film thickness (Agilent Technologies; Mississauga, Ontario, Canada). The limit of detection (LOD) for total BPA in urine was 0.2 µg/L; for free BPA in urine it was 0.012 µg/L. The LOD for total TCS in urine was 0.12 µg/L; for free TCS in urine it was 0.008 µg/L. The LOD was calculated by using the value equivalent to three times the standard deviation of 10 replicates of a sample at a concentration from 4 to 10 times the estimated LOD with a signal to noise ratio (S/N) of 3.

Enzymatic hydrolysis was used to deconjugate phthalate metabolites (88). Phthalate monoester compounds were extracted by solid phase extraction with anion exchange media using the Janus robotic station (Perkin Elmer; Waltham, Massachusetts, USA). The extracts were dried, taken up by water and analyzed by LC-MS/MS with an Ultra Performance Liquid Chromatography (UPLC) Acquity (Waters; Milford, Massachusetts, USA) in MRM mode with an electroscopy ion source in negative mode. The analytic column used was an Acquity BEH Phenyl with a 50 mm x 2.1 mm i.d. x 1.7 µm film thickness (Waters; Milford, Massachusetts, USA). The LOD for the phthalate monoester metabolites ranged from 0.2 to 5.0 µg/L. They were also estimated as a function of the S/N of 3, however this was done in real samples as most of the phthalate monoesters had concentrations too high in normal urine to be calculated with the standard deviation, as was conducted for the BPA and TCS urine samples.

To ensure consistency and conformity amongst all the samples, the laboratory provided all biospecimen containers. Further, to assess potential risk of contamination

during the collection, processing, transportation and storage of the urine samples, all containers were tested and field blanks were employed using Steril.O deionized distilled water as the sampling medium. Analyses of the field blanks were conducted using the same procedures for the analysis of the urine samples. Additionally, as a measure of hydration status (urine dilution), specific gravity of the urine was measured by refractometry (Atago UG-α, Cat. # 3464) with automatic temperature compensation.

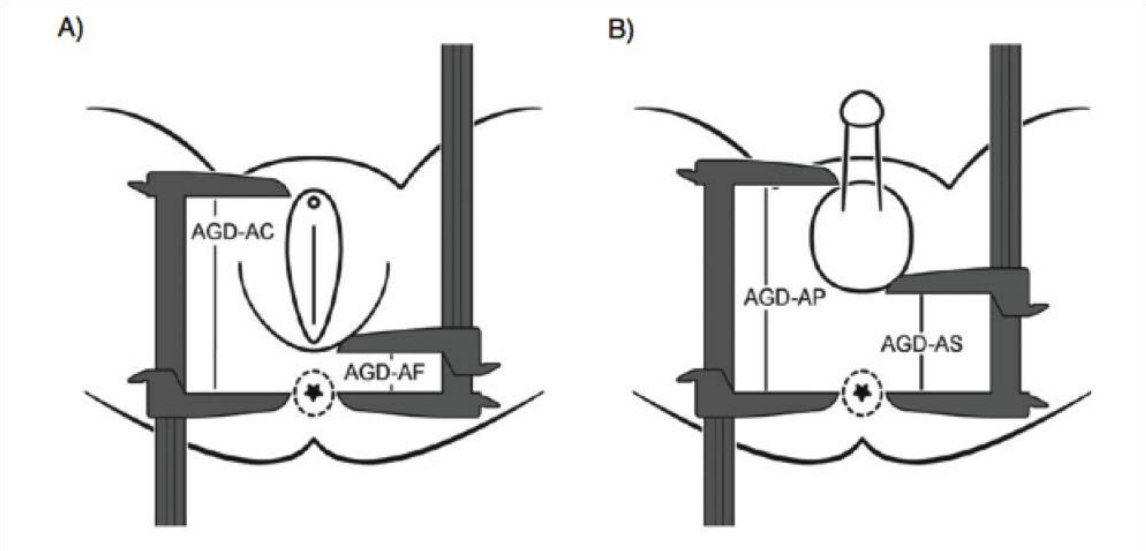
Furthermore, for each participant, several sampling characteristics were noted at the time of urine collection, including: the date and time it was collected and the number of hours since last urination. The date and time the urine sample was frozen and placed in the MIREC Biobank was also recorded. All samples were labelled with a unique ID and barcode, similar to the questionnaire data.

3.7 Outcome measurements

Measures of sexual and reproductive development represented by the anogenital distance were obtained at birth (24-48 hours post-delivery) as part of the MIREC-ID study. For female infants, the distance (in mm) from the center of the anus to the posterior convergence of the fourchette (anus-fourchette) or the clitoris (anus-clitoris distance) was measured. Two measurements were taken and reported; if there was a greater than 2 mm difference between the 2 measures, then a third measurement was also taken. If the measurement could not be performed, the reason was recorded. To measure both distances in female infants, the infant was required to be in the dorsal decubitus position. Similarly, for male infants, the distances (in mm) between the base of the scrotum and the mid-anus (anoscrotal distance) and between the centers of the anus to the

cephalad base of the penis (anogenital distance) were measured. It was ensured that the scrotal sac was not lifted away from the measurement area to avoid over-estimation. For the measurement of the distances, metric dial vernier calipers (Scienceware; Bel-Art Products, Pequannock, New Jersey) with modified rounded corners with increments of 0.1 mm were used. The calliper was properly calibrated and set to zero prior to each measurement. The female and male distances measured are shown in diagram A and B, respectively (Figure 1). For the purpose of this analysis, the mean of the 2 closest measures was calculated and used.

FIGURE 1: ANOGENITAL DISTANCE MEASUREMENTS IN FEMALE (A) AND IN MALE (B) INFANTS

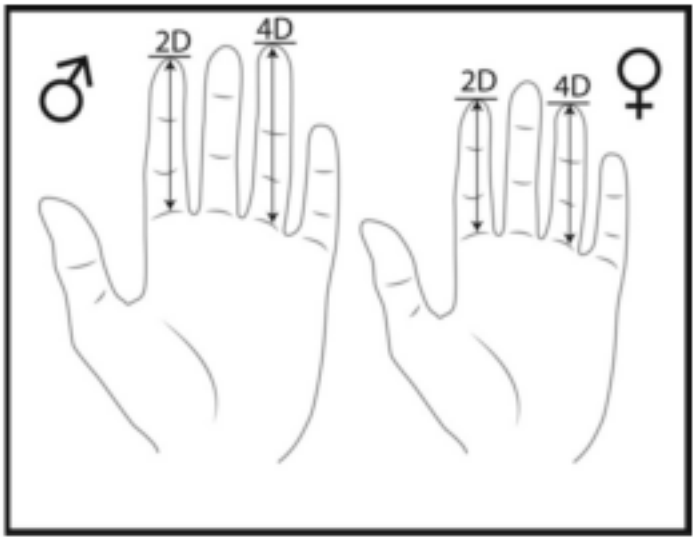


Sathyanarayana, S., Beard, L., Zhou, C., & Grady, R. (2010). Measurement and correlates of ano-genital distance in healthy, newborn infants. *International Journal of Andrology*, *33*(2), 317–23. (89)

A second outcome of interest for this study was the ratio of the lengths of the second and fourth finger digits (2D:4D digit ratio). The length (in cm) of the second and fourth finger of both the left and right hand were measured during the 6-month assessment of the infant. These measurements were done with a transparent plastic ruler with millimetre increments, assuring the bottom of the ruler was aligned with the basal

crease of each finger. Measurements were taken twice for each finger length for each hand. For the purpose of this analysis, the mean finger length for each hand was employed.

FIGURE 2: MEASUREMENT OF THE SECOND AND FOURTH DIGITS IN MALE AND FEMALE INFANTS



Dean, A., & Sharpe, R. M. (2013). Anogenital distance or digit length ratio as measures of fetal androgen exposure: relationship to male reproductive development and its disorders. *The Journal of Clinical Endocrinology and Metabolism*, *98*(6), 2230–8. (90)

3.8 Definition of Potential Risk Factors (Confounding Variables)

The independent variables or exposures of interest for this thesis project are the urinary concentrations of BPA, TCS and phthalates (µg/L) in pregnant women, as measured by single spot urine samples. Additionally, combined phthalate exposure values were also calculated based on the molar sums of individual phthalate metabolites; this is explained in detail in a later section.

The covariates that were considered in all study models included several demographic variables, anthropometric measurement variables and urine collection parameters, many of which were derived. Variables were chosen based on numerous

studies demonstrating variation in reproductive outcomes depending on maternal characteristics and various urine collection parameters. Maternal active and passive smoke exposure was also included as smoking during pregnancy and exposure to environmental tobacco smoke (ETS) has been known to be associated with many adverse events, including altered reproductive development in males (91). Prenatal exposure to nicotine (the active component in cigarettes) significantly reduced AGD at birth in female and male mice (92). A study in humans found that maternal smoking and exposure to ETS resulted in an increased anogenital index (13). Additionally, it may also be associated with feminization of 2D:4D finger digit ratio in males, as tobacco smoke is known to affect masculinization by altering fetal testosterone levels (91,93).

The following variables determined during visit 1 of the MIREC study were derived:

- **Maternal age**, determined from the following questions:
    1) What is today's visit date? (MIREC participant visit 1 date)
    2) What is your year of birth?

The participant visit date at visit 1 of the MIREC study was subtracted from a set date of June 30th and the participant year of birth; this value was then divided by 365.25 to get a range of possible birth dates for all participants. For the analysis, maternal age was kept as a continuous variable. For the descriptive analysis, maternal age was categorized as follows: 1=<25 years, 2=25-29 years, 3=30-34 years, 4=≥35 years.

- **Maternal education**, determined from the following question:

1. Highest education level achieved? (Grade 8 or less/some high school/high school diploma/some college classes/college diploma/trade school diploma/undergraduate university degree/graduate university degree)

For the analysis, maternal education level categories were collapsed as follows: 1=≤High School Diploma, 2=Some College, 3=College or Trade Diploma, 4=University Degree.

- **Maternal race**, determined from the following question:
    1. People in Canada come from many racial or cultural groups. You may belong to more than one group on the following list. Are you…? (White/Chinese/South Asian/Black/Filipino/Southeast Asian/Latin American/Arab/West Asian/Japanese/Korean/Aboriginal/Other/If other, specify/refuse to answer/don't know)

For the analysis, maternal race categories were collapsed as follows: 1=White, 2=Other. The basis for choosing 'White' and 'Other' as the categories for this variable was because the study population was predominantly White.

- **Maternal country of birth**, determined from the following question:
    1. In what country were you born? (Canada/United States/Mexico/China/Other/If other, specify/don't know)

For the analysis, maternal country of birth categories were collapsed as follows: 1=Canada, 2=Other. The basis for choosing 'Canada' and 'Other' as the categories for this variable was because the study population was predominantly from Canada. In most cases, there were less than 5 mothers born in other specific countries.

- **Maternal pre-pregnancy body mass index (BMI)**, determined from the following questions:
    1. Pre-pregnancy weight? (Kg/Pounds, don't know)
    2. Height measured at this visit (cm)

For the analysis, BMI was kept as a continuous variable. For the descriptive analysis, maternal BMI was categorized into the following categories: 1=<18.50 kg/m^2, 2=18.50 kg/m^2 -24.99 kg/m^2, 3=25 kg/m^2-29.99 kg/m^2, 4=>30 kg/m^2

- **Maternal marital status**, determined from the following question:
    1. Marital status? (Married/same partner for 1 year or more/widowed/divorced/separated/single/other/If other, specify)

For the analysis, maternal marital status categories were collapsed as follows: 1=Married, 2=Separated, 3=Divorced, 4=Single, 5=Other/Unknown.

- **Annual household income**, determined from the following question:
    1. From all sources from Jan-Dec last year, what was your annual household income before taxes? (Including other sources of income, help from family or friends) (Less than $10,000/$10,001-$20,000/$20,001-$30,000/$30,001-$40,000/$40,001-$50,000/$50,001-$60,000/$60,001-$70,000/$70,001-$80,000/$80,001-$100,000/More than $100,000/don't know/refuse to answer)

For the analysis, annual household income categories were collapsed as follows: 1=≤$20,000, 2=$20,001-$40,000, 3=$40,001-$60,000, 4=$60,001-$80,000, 5=$80,001-$100,000, 6=>$100,000.

- **Active smoke exposure**, determined from the following questions:
    1. Have you ever smoked at least 100 cigarettes over your lifetime (about 4 packs)? (yes/no)
    2. At the present time, do you smoke cigarettes daily, occasionally or not at all? (daily/occasionally/not at all)

For the analysis, active smoke exposure was categorized as follows: 1=Prior to Pregnancy, 2=Occasionally during Pregnancy, 3=Daily during Pregnancy, 4=Never. If the participant had answered 'yes' to the first question and 'not at all' to the second question, they were considered past smokers and were categorized in the 'prior to pregnancy' group.

- **Passive smoke exposure**, determined from the following questions:
    1. Including both household members and regular visitors, does anyone smoke inside your home, every day or almost every day? (Note: include cigarettes, cigars and pipes) (yes/no/don't know/refuse to answer)
    2. In the past month, were you exposed to second-hand smoke every day, or almost every day, in a car or other private vehicle? (yes/no/don't know/refuse to answer)
    3. During your pregnancy, has anyone in your workplace smoked in your presence? (Including breaks, lunch) (yes/no/not applicable/don't know/refuse to answer)
    4. During your pregnancy, were you exposed to second-hand smoke in public places? (such as bars, arenas, restaurants or bingo halls) (yes/no/don't know/refuse to answer)

For the analysis, passive smoke exposure was categorized as follows: 1=Yes, 2=No. If the participant answered 'yes' to any of the four questions, they were considered to be passively exposed to cigarette smoke.

- **Season of urine collection**, determined from the following question:
    1. Date of sample urine collection?

For the analysis, the season of urine collection was categorized as follows: 1=Spring, 2=Summer, 3=Autumn, 4=Winter. Spring was defined as March 20th – June 21st; summer was defined as June 22nd – September 22nd; autumn was defined as September 23rd – December 21st; and winter was defined as December 22nd – March 19th.

- **Time of urine collection**, determined from the following question:
    1. Time of sample urine collection? (hours and minutes)

For the analysis, the time of urine collection was categorized as follows: 1=6:00-9:00, 2=9:00-12:00, 3=12:00-15:00, 4=15:00-18:00, 5=18:00-21:00, 6=21:00-24:00.

- **Time since last urine void**, determined from the following question:
    1. Number of hours since last urination? (prior to this one) (hours and minutes)

For the analysis, the time since last urine void was categorized as follows: 1=Fewer the 75 minutes, 2=76-120 minutes, 3=121 minutes – 170 minutes, 4=>170 minutes.

All questions were taken from the case-report forms (CRFs). In the case of categorical variables, all categories were ensured to have a minimum sample size of 5 participants.

Several other variables were included in the analysis. Specific gravity of the urine was included in the analysis to account for the dilution of the urine; this was measured and included in the biomonitoring data.

Furthermore, infant weight and length at birth and at the 6-month assessment, measured as part of the MIREC-ID study were also included. As was the case with AGD, initially two measures for weight and length were obtained. If the two measures for infant weight differed by greater than 5 g, then a third measurement was taken. Similarly, if the two measures for infant length differed by greater than 3 mm, then a third measurement was taken. The mean of the two measurements was calculated and used for this analysis; if a third measurement was taken, then the mean of the 2 closest measures was calculated and used. Additionally, to account for the age of the infant, the gestational age at delivery measured at birth during the MIREC study and the infant age at the 6-month assessment were included. The infant age at the 6-month assessment was calculated by subtracting the delivery date obtained from the MIREC study from the visit date obtained from the MIREC-ID study. Lastly, a weight-for-length z score was calculated based on the standards set out by World Health Organization (94); this was done to appropriately account for the age and body size of the infant without having variables that were highly inter-correlated.

All results were stratified by the gender variable (1=Male Infant, 2=Female Infant). Gender data was obtained from the MIREC-ID dataset. If it was not available, then it was extracted from the MIREC dataset. Any data that was missing was considered a uniform non-response (i.e. missing completely at random, MCAR). Although there is a

way to test if data is MCAR (i.e. a likelihood ratio test), this was not done as part of the analysis.

3.9 Data cleaning

This thesis project consisted of the analysis of secondary datasets, obtained from the MIREC research platform.

Of the datasets included for this thesis, one contained the biomonitoring data for all biospecimens included in the MIREC biobank. The dataset included the laboratory measurement of each environmental chemical metabolite for each participant, which was measured as a concentration in the respective biospecimen and identified by unique participant identification numbers. It also contained the unit of measurement for each metabolite, measurement date, the center the participant was associated with (of total 10 centers), the visit number and the urine aliquot number. This dataset also contained measurements of specific gravity and creatinine, for urine concentration correction purposes. Cleaning of this dataset included the assessment of the laboratory results, which included identifying missing data (which may have been due to damaged specimens, insufficient quantity of specimen for testing, other reasons) and the identification of values below the LOD (application of LOD/2). Further, as the laboratory data did not meet the assumptions for normality, it was logarithmically transformed (see data analysis section for a detailed explanation). A final dataset containing only laboratory data pertaining to the environmental chemicals of interest for this thesis project was obtained.

Multiple small datasets contained all the demographic information collected from the participant questionnaires conducted during visit 1 (first trimester) of the MIREC study. These datasets included baseline data (e.g. maternal birth year, education, income, marital status, country of birth, ethnicity, estimated gestational age of the baby); anthropometric measurements (e.g. pre-pregnancy weight and height at current visit); urine collection data (e.g. date and time of sample collection, time since last urination); smoking history data and exposure to second hand smoke data. Additionally, there was a dataset containing all the information collected during the post-delivery neonatal visit, which included birth weight, birth length, gender and gestational age at birth. Along with the patient identification numbers, each dataset also contained the visit number, visit date and center for each participant. As part of the data cleaning process, several derived variables were created as identified in section 3.8.

Additionally, multiple small datasets contained data collected from the MIREC-ID study. This included data on the two outcome variables (e.g. anogenital distance measurements measured at birth and the measurement of the length of the second and fourth digit of both the right and left hands measured at 6-months) for all infants, in addition to birth information data (e.g. gender, gestational age at birth and delivery date) and anthropometric measurement data (e.g. birth weight, birth length, weight at the 6-month assessment and the length at the 6-month assessment).

All variables and observations in the MIREC and MIREC-ID datasets were thoroughly checked and cleaned. Each variable was identified as either continuous or categorical. Continuous variables were checked against a normal distribution, while categorical variables were ensured to have a minimum of 5 entries per category. Further,

data cleaning included looking for any gaps, inconsistencies, outliers and missing information. There was a presence of several outliers for select measurement variables; upon consultation with study personnel at the MIREC coordinating centre, it was determined that the units of measurement were incorrectly identified. Further, select variables were present in both the MIREC and MIREC-ID datasets, such as gender, birth weight and birth length; these were checked to ensure consistent reporting of results. If there was any discrepancy, the appropriate action was taken upon consultation with study personnel at the coordinating centre and Health Canada.

All laboratory, MIREC and MIREC-ID datasets were then merged into two final datasets; the first dataset contained all the participants for which there was MIREC-ID birth assessment data available (named the 'birth cohort') and the second dataset contained all the participants for which there was MIREC-ID 6-month assessment data available (named the '6-month cohort'). The final datasets contained the variables as defined in the 'definition of variables' section above. These datasets were then prepared for analysis using Statistical Analysis Software (SAS) 9.3 (SAS Institute, Cary, NC).

3.10 Data Analysis

This thesis project entailed the analysis of data collected from the MIREC and MIREC-ID studies. This included administered questionnaires, endocrine sensitive endpoint measures and maternal urine specimen results from the INSPQ lab. All MIREC and MIREC-ID datasets were imported from DACIMA – an online tool used to manage large datasets (Dacima Software, Montreal, Quebec) – into Statistical Analysis Software (SAS) 9.3 (SAS Institute, Cary, NC).

3.10.1 Limits of detection

In biomonitoring studies, when the concentration of a chemical is less than the laboratory's detection or reporting limit for that specific chemical, the value is referred to as below the 'limit of detection' or 'censored data'. There are multiple ways to account for values below the LOD in a statistical analysis; these include substituting zero for the value, the LOD itself or dividing the LOD by 2 or the square root of 2. There is evidence that these substitution methods may be biased (known to artificially reduce the standard error), with the recommendation that a censoring method be used (95), where the characteristics of the distribution of the values above the LOD are used to estimate the values below the LOD. Censoring methods include parametric testing (where distributional assumptions are made and a maximum-likelihood test is used) and non-parametric testing (where Kaplan Meier curves are created). Another method commonly used to deal with values below the LOD is called the multiple imputation method.

In this analysis, all contaminants for which greater than 50% of the samples were below the LOD were excluded from further analysis. All other contaminants had a relatively low number of samples below the LOD (i.e. <20% for the majority of contaminants). As the overall percentage of samples below the LOD was small for the included contaminants, the LOD/2 was substituted for values below the LOD. Other studies conducted in this field of research have used the same approach to deal with values below the LOD.

3.10.2 Dilution of Urine

The concentration or dilution of urine is highly dependent on how hydrated the body is and whether one has engaged in activity/exercise, among other factors (96). There are two methods that are commonly used to adjust for this, creatinine (CR) correction and specific gravity (SG) correction. Creatinine is a by-product of muscle activity that is excreted in urine by the renal system. Specific gravity is the ratio of the density of urine in comparison to the density of water. Though CR correction has been the method of choice historically, SG correction is recently a more widely used tool, especially for pregnant women, as it limits biases that may exist otherwise (i.e. intra-day variation in creatinine measurements) (96–98).

Urinary concentrations were adjusted for specific gravity using the following formula:

$$P_c = P_i\,[(SG_m - 1)/(SG_i - 1)]$$

where P_c is the specific gravity adjusted concentration of the metabolite, P_i is the observed concentration of the metabolite, SG_m is the median specific gravity for the cohort and SG_i is the specific gravity of the urine sample. Both unadjusted and specific gravity adjusted concentrations were reported for the descriptive statistics of the urinary contaminants. For the purposes of the main objective, all statistical analyses conducted considered specific gravity adjusted metabolite concentrations by including specific gravity as a covariate in the model. The inclusion of the variable in the model allows for the urinary concentration of the chemical to be properly adjusted (82).

3.10.3 Descriptive Analysis

Descriptive statistics were used to describe the study population demographically, the population in relation to the parameters surrounding urine collection and the maternal urinary contaminants. These statistics were applied to both the birth cohort and the 6-month cohort. To begin, the study population was described using demographic variables applicable to the pregnant women (i.e. the mother); these included: age, education, marital status, household income, country of birth, pre-pregnancy body mass index, active smoking status and passive smoking status. The infants in the population were described by gender, mean birth weight, mean weight at the 6-month assessment, mean anogenital distance (all 4 measures) and mean 2D:4D digit ratio. Moreover, descriptive statistics were employed to describe the study population as it applies to the urine collection parameters, including: season of collection, time of collection and time since last urine void. Lastly, all contaminants (i.e. 11 phthalate metabolites, total and free BPA and TCS) were described using descriptive statistics. The following was calculated and recorded for each contaminant: the total number of urine samples detected with the specific contaminant (i.e. sample size), the minimum and maximum concentration, the overall geometric mean concentration, the 95% confidence interval, the sample median concentration and the sample 95% percentile. Further, as part of the descriptive analysis of the contaminant data, the LOD was recorded for each contaminant in addition to the total percentage of samples that were below the LOD; the LOD for each contaminant was provided by the INSPQ laboratory.

3.10.4 Variations in maternal non-occupational exposure to select phthalate metabolites, BPA and TCS

Descriptive summary statistics were calculated for urinary concentrations of select phthalate metabolites, BPA and TCS to evaluate inter-individual variability in the study population. Further, the geometric mean urinary concentrations of these chemicals were compared between mothers of White race versus those of other races. These included mothers of the Chinese, South Asian, Black, Filipino, Southeast Asian, Latin American, Arab, West Asian, Japanese, Korean and Aboriginal races. Any significant differences were tested using an independent two-sample t-test.

3.10.5 Association between maternal non-occupational exposure to select environmental contaminants and the anogenital distance and 2D:4D finger digit ratio in infants

The main purposes of this thesis project was to determine whether maternal exposure to BPA, TCS or select phthalate metabolites is associated with the changes in the anogenital distance and the 2D:4D digit ratio in the infant. Following the initial descriptive analysis, as mentioned earlier, upon consultation with study personnel, all contaminants for which greater than 50% of the samples were below the LOD were excluded from further analysis. Therefore, the predictors of interest (i.e. independent variables) were identified to be: MBP, MBzP, MCPP, MEHHP, MEHP, MEOHP, MEP, total BPA, total TCS and free or unconjugated TCS. The outcome variables included both the short anogenital distance measure (i.e. the anus-fourchette and the anus-scrotal distances in females and males, respectively), the long anogenital distance measure (i.e. the anus-clitoris and the anus-cephalad base of the penis in females and males,

respectively) and the 2D:4D digit ratio in both the left and right hands. As mentioned, all results were stratified by gender, as gender may be an effect-modifying variable, as mentioned in earlier literature.

The initial analysis suggested that the contaminant or exposure data was positively skewed and thus unable to meet one of the main assumptions for parametric testing, based on results of the Shapiro-Wilk test. Further, a residual analysis demonstrated there was extreme heteroscedasticity. The log transformation of exposure data has been done in almost all AGD studies, and is thus, widely accepted as the norm Thus, all contaminant data were log-transformed and included as such in all the statistical models. To begin, simple linear regression models (i.e. univariate linear regression) were used to examine the relationship between infant AGD and 2D:4D digit ratio and each of the risk factor variables (including the exposure of interest and all covariates) independently (i.e. potential confounders). These variables included: maternal age, education, marital status, country of birth, race, passive smoke exposure, active smoke exposure, pre-pregnancy BMI, gestational age at delivery (for birth cohort), age at 6 month visit (for 6-month cohort), gender of the baby, infant birth weight and length (for birth cohort), weight at 6-month visit and length (for 6-month cohort), weight-for-length z-score and 3 urine collection parameters (season of urine collection, time or urine collection and time since last urination). Although many of these variables may not be obvious ‘risk factors’ of the outcomes, they were included as part of an exploratory analysis and to better understand the data at hand. Any variables that were associated with anogenital distance or the 2D:4D digit ratio at $P \leq 0.15$ were recorded.

Subsequently, multiple linear regression models were created to examine the relationship between the anogenital distance and the 2D:4D digit ratio and the exposure of interest, including specific gravity and significant covariate variables as identified from the univariate analysis. To determine the best model, stepwise selection was used with entry and removal of variables at $p \leq 0.05$. It should be noted that there are many different approaches to building a regression model – the use of stepwise regression is just one of these approaches. The final model was checked to be significant overall, based on the F-statistic and the associated p-value.

Prior to making any definite conclusions, all models were thoroughly checked through the process of diagnostic testing. This included checking for any observations that had a high influence on the overall model (using Cook's D statistic and dfbeta plots) or high leverage in relation to the parameter estimates specifically. Once again, the error terms or residual values were checked to ensure they were normally distributed. This was illustrated by plotting a normal probability curve and quantile-quantile plots.

To examine the effects of a combined exposure to a number of the phthalate metabolites on the anogenital distance and the 2D:4D digit ratio, the concentration of individual metabolites were summed together to create one exposure risk variable. Individual metabolites were grouped according to use. As mentioned earlier, low-molecular weight phthalates are typically used in cosmetics, lotions and other personal care products (99,100), while high-molecular weight phthalates are predominantly used as plasticizers (101). Thus, all low molecular weight phthalates were considered as one entity, while all high molecular weight phthalates were considered as another. Low molecular weight phthalates included the following metabolites: MMP, MEP, MBP,

MCHP, MCPP, and MBzP. Alternatively, high molecular weight phthalates included the following metabolites: MEHP, MOP, MEOHP, MNP, and MEHHP. To sum the concentrations for all low and high phthalate metabolites, each result was converted to a molarity by dividing each value by its respective molecular weight (g/mol). Upon calculating a sum concentration for all low and high molecular weight phthalates, multiple linear regression models were created. Models were created and tested in a similar manner to the aforementioned methodology.

3.10.6 Correlation between the anogenital distance and the 2D:4D digit ratio

To test for the presence of a correlation between the two outcome measures of interest, anogenital distance and 2D:4D digit ratio, a Spearman correlation test was conducted. As the variables were not normally distributed, non-parametric testing was the most ideal methodology to employ.

CHAPTER 4: RESULTS

4.1 Descriptive Statistics: Birth Cohort

The study population in the birth cohort consisted of 370 mother-infant pairs (for the phthalate metabolites), 396 mother-infant pairs (for total BPA) and 394 mother-infant pairs (for total and free TCS). The total sample size decreased from the initial sample size of 396 mother-infant pairs (for the phthalate metabolites and total/free TCS) due to the exclusion of participants with missing contaminant data. Table 3 describes this population by various demographic categories and urine collection parameters. Among the 370 mothers (sample size used for descriptive statistics purposes), 142 (38%) were between the ages of 30 and 34 years, 206 (56%) had a pre-pregnancy body mass index between 18.50-24.99 kg/m^2, 91 (25%) had a college/trade school diploma while 227 (61%) had a university degree, 113 (31%) had a household income greater than $100,000, 248 (67%) were married and 317 (86%) were born in Canada. In regards to exposure to environmental tobacco smoke, 105 (28%) of women had actively been exposed to tobacco smoke prior to pregnancy, while 178 (48%) of women had passively been exposed to tobacco smoke during pregnancy. There were 24 (6%) women who smoked during pregnancy (either occasionally or daily). Most urine collection occurred in the fall season (34% of women), between the times of 09:00-12:00 (51% of women). Most women had last urinated 76-120 minutes ago (28%) or greater than 170 minutes ago (28%).

There was a relatively even distribution of male and female babies in this cohort, 49.19% and 50.81%, respectively. On average, babies weighed 3.34 kg and had a birth

length of 50.47 cm. As illustrated in Figure 3, in females, the mean anus-clitoris distance was approximately 33.51 mm, while the mean anus-fourchette distance was much smaller at 14.50 mm. On the other hand, in males, the mean anogenital distance was 43.86 mm, while the mean anoscrotal distance was 22.52 mm.

TABLE 3: DEMOGRAPHIC COVARIATES OF A SAMPLE OF MIREC AND MIREC-ID PARTICIPANTS (BIRTH COHORT) PROVIDING 1ST TRIMESTER URINE SAMPLES FOR ANALYSIS OF PHTHALATE METABOLITES, BPA AND TCS (N=370)

	Frequency	Percentage (%)
Maternal Age (years)		
<25	17	4.59
25-29	98	26.49
30-34	142	38.38
≥35	113	30.54
Maternal Education		
≤High School	33	8.92
Some College	17	4.59
College/Trade School Diploma	91	24.59
University Degree	227	61.35
Missing	2	0.54
Household Income ($)		
≤20,000	22	5.95
20,001-40,000	26	7.03
40,001-60,000	51	13.78
60,001-80,000	69	18.65
80,001-100,000	81	21.89
>100,000	113	30.54
Missing	8	2.16
Mother's Marital Status		
Married	248	67.03
Separated	1	0.27
Divorced	1	0.27
Single	14	3.78
Other/Unknown	106	28.65
Mother's Country of Birth		
Canada	317	85.68
Other	53	14.32
Mother's Race		
White	333	90.00
Other	37	10.00
Mother's Smoking Status (Active)		
Prior to pregnancy	105	28.38
Occasionally during pregnancy	8	2.16
Daily during pregnancy	16	4.32
Never	241	65.14
Mother's Exposure to Environmental Tobacco Smoke (Passive)		
Yes	178	48.11
No	192	51.89
Pre-pregnancy Body Mass Index (kg/m^2)		
≤18.50	5	1.35

18.50-24.99	206	55.68
25.00-29.99	69	18.65
>30	62	16.76
Missing	28	7.57
Season of Urine Collection		
Spring	91	24.59
Summer	80	21.62
Fall	127	34.32
Winter	72	19.46
Time of Urine Collection		
6:00-9:00	7	1.89
9:00-12:00	187	50.54
12:00-15:00	112	30.27
15:00-18:00	62	16.76
18:00-24:00	2	0.54
Time Since Last Urination (min)		
≤75	93	25.14
76-120	108	29.19
121-170	55	14.86
>170	102	27.57
Missing	12	3.24
Gender of Baby		
Male	182	49.19
Female	188	50.81
Mean Birth weight (kg)	3.34	
Mean Birth length (cm)	50.47	

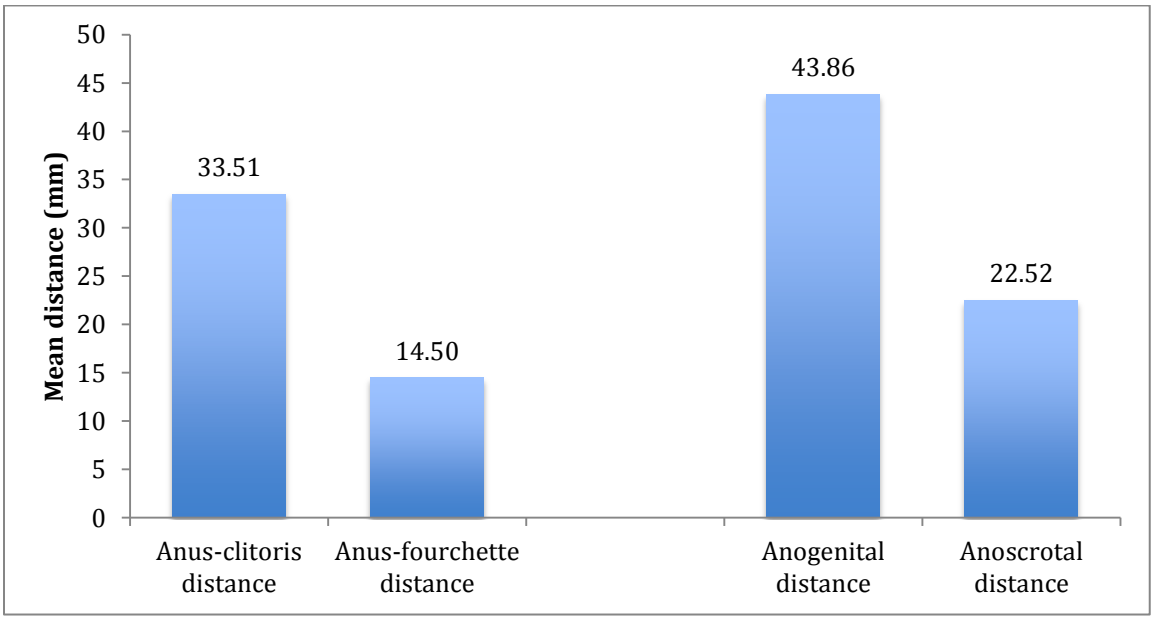


FIGURE 3: MEAN ANOGENITAL DISTANCES OBSERVED IN A SAMPLE OF MALE AND FEMALE INFANTS FROM THE MIREC AND MIREC-ID STUDY

Table 4 reports the overall summary statistics (sample size, minimum, maximum, geometric mean with 95% lower and upper confidence intervals, sample median and

sample 95% percentile) for each contaminant both unadjusted and specific-gravity adjusted, detected from urine samples in mothers in the birth cohort. The first entry in the table represents the unadjusted statistics (e.g. MnBP), while the second entry represents the specific gravity adjusted statistics (e.g. MnBP_adj). The column labelled 'LOD' reports the LOD for each contaminant, while the %<LOD reports the proportion of observations below this value. All values below the LOD were substituted using the ½ LOD substitution methods. Further, any values reported below the LOD, were reported. It should be noted that the overall number of observations for total BPA and TCS in addition to free BPA and TCS were higher than that of individual phthalate metabolites due to differences in the number of aliquots of urine available for testing by individual.

TABLE 4: SUMMARY OF CONTAMINANTS FOUND IN URINE SAMPLES OF PARTICIPANTS FROM THE MIREC/MIRED-ID STUDY (BIRTH COHORT)

Contaminant (µg/L)	N	LOD	%<LOD	Min	Max	Geometric Mean	95% Confidence Interval		Sample Median	Sample 95th Percentile
							Lower	Upper		
Specific Gravity	396	.	0.00	1.00	1.03	1.01	1.01	1.01	1.01	1.01
MnBP	370	0.20	0.00	0.36	800.00	10.66	9.43	12.05	12.05	12.00
MnBP_adj	370	0.20	0.00	0.45	800.00	12.92	11.92	14.02	14.02	12.54
MBzP	369	0.20	0.54	ND	230.00	5.15	4.52	5.86	5.86	5.40
MBzP_adj	369	0.20	0.54	ND	312.00	6.20	5.59	6.88	6.88	5.72
MCHP	370	0.20	94.59	ND	77.00	ND	ND	ND	ND	ND
MCHP_adj	370	0.20	94.59	ND	47.67	ND	ND	ND	ND	ND
MCPP	370	0.20	18.65	ND	26.00	0.73	0.63	0.84	0.84	0.83
MCPP_adj	370	0.20	18.65	ND	36.83	0.88	0.79	0.99	0.99	0.86
MEHHP	370	0.40	1.62	ND	520.00	7.61	6.72	8.64	8.64	8.35
MEHHP_adj	370	0.40	1.62	ND	355.79	9.23	8.51	10.02	10.02	9.10
MEHP	366	0.20	1.37	ND	110.00	1.95	1.74	2.18	2.18	2.05
MEHP_adj	366	0.20	1.37	ND	52.96	2.33	2.15	2.53	2.53	2.21
MEOHP	370	0.20	0.81	ND	290.00	5.39	4.78	6.08	6.08	6.00
MEOHP_adj	370	0.20	0.81	ND	171.05	6.54	6.06	7.05	7.05	6.50
MEP	370	0.50	0.27	ND	13000.00	27.15	23.21	31.75	31.75	25.00
MEP_adj	370	0.50	0.27	ND	9388.89	32.92	28.64	37.83	37.83	27.80
MMP	370	5.00	83.24	ND	87.00	ND	ND	ND	ND	ND
MMP_adj	370	5.00	83.24	ND	53.86	ND	ND	ND	ND	ND
MiNP	370	0.40	98.38	ND	6.20	ND	ND	ND	ND	ND
MiNP_adj	370	0.40	98.38	ND	2.99	ND	ND	ND	ND	ND
MnOP	370	0.70	97.57	ND	1.10	ND	ND	ND	ND	ND
MnOP_adj	370	0.70	97.57	ND	4.55	ND	ND	ND	ND	ND
Total BPA	396	0.20	12.12	ND	17.00	0.78	0.69	0.87	0.87	0.82
Total BPA_adj	396	0.20	12.12	ND	21.67	0.90	0.82	0.99	0.99	0.82
Free BPA	394	0.012	59.64	ND	1.24	ND	ND	ND	ND	ND
Free BPA_adj	394	0.012	59.64	ND	0.61	ND	ND	ND	ND	ND
Total TCS	394	0.12	1.52	ND	2322.96	10.28	8.21	12.86	12.86	7.67
Total TCS_adj	394	0.12	1.52	ND	2745.32	11.92	9.58	14.82	14.82	7.53
Free TCS	394	0.008	23.10	ND	36.71	0.07	0.05	0.08	0.08	0.05
Free TCS_adj	394	0.008	23.10	ND	20.75	0.08	0.06	0.09	0.09	0.05

For each contaminant, Figure 4 illustrates the proportion of observations that are below the LOD. All contaminants for which over 50% of observations were below the LOD, were omitted from the analysis due to uncertainty in the data.

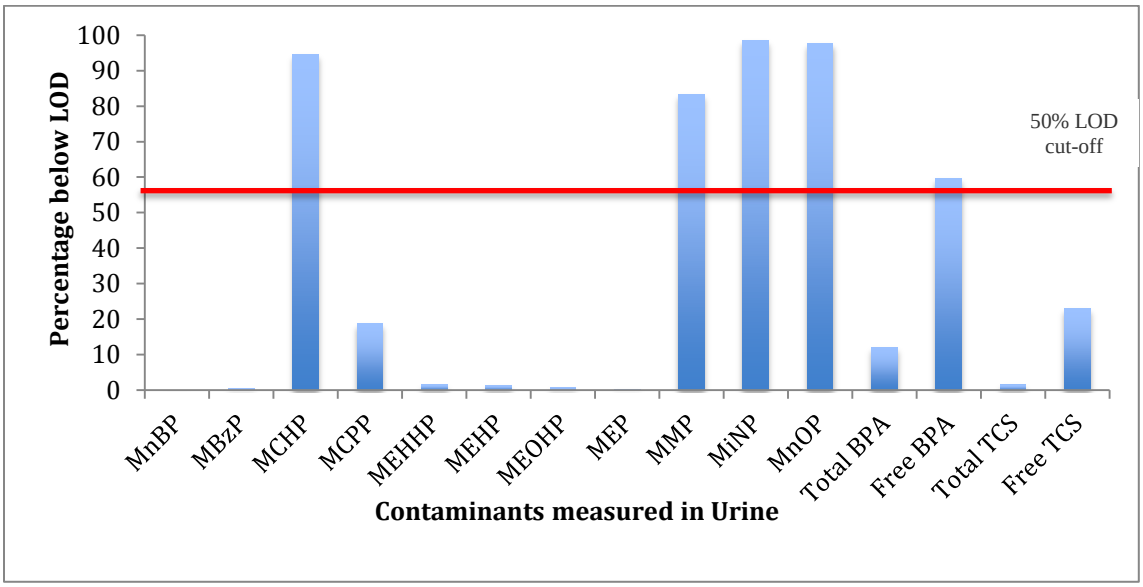


FIGURE 4: PROPORTION OF OBSERVATIONS BELOW LOD FOR MEASURED URINE CONTAMINANTS

4.2 Descriptive Statistics: 6-month cohort

The study population in the 6-month cohort consisted of 393 mother-infant pairs (for the phthalate metabolites), 418 mother-infant pairs (for total BPA) and 417 mother-infant pairs (for total and free TCS). The total sample size decreased from the initial sample size of 418 mother-infant pairs (for the phthalate metabolites and total/free TCS) due to the exclusion of participants with missing contaminant data. Table 5 describes this population by various demographic categories and urine collection parameters. The descriptive statistics results are similar to that of the birth cohort (as many mother-infant pairs were in both datasets); however, they are not the same as some mother-infant pairs may have missed the birth assessment during MIREC-ID. Among the 390 mothers, 147 (37%) were between the ages of 30 and 34 years, 212 (54%) had a pre-pregnancy body

mass index between 18.50-24.99 kg/m^2, 87 (22%) had a college/trade school diploma while 261 (66%) had a university degree, 136 (34%) had a household income greater than $100,000, 275 (70%) were married and 337 (86%) were born in Canada. In regards to exposure to environmental tobacco smoke, 113 (29%) of women had actively been exposed to tobacco smoke prior to pregnancy, while 185 (47%) of women had passively been exposed to tobacco smoke during pregnancy. There were 19 (3%) women who smoked during pregnancy (either occasionally or daily). Most urine collection occurred in the fall season (31% of women), between the times of 09:00-12:00 (50% of women). Most women had last urinated 76-120 minutes ago (28%) or greater than 170 minutes ago (26%).

There was a relatively even distribution of male and female babies in this cohort, 50.64% and 49.36%, respectively. On average, babies weighed 7.97 kg at 6-months and had a length of 68.04 cm. The mean 2D:4D digit ratio in the left hand was 0.94 and the mean 2D:4D digit ratio in the right hand was 0.95.

TABLE 5: DEMOGRAPHIC COVARIATES OF MIREC AND MIREC-ID PARTICIPANTS (6-MONTH COHORT) PROVIDING 1ST TRIMESTER URINE SAMPLES FOR ANALYSIS OF PHTHALATE METABOLITES, BPA AND TCS (N=393)

	Frequency	Percentage (%)
Maternal Age (years)		
<25	15	3.82
25-29	89	22.65
30-34	147	37.40
≥35	142	36.13
Maternal Education		
≤High School	26	6.62
Some College	17	4.33
College/Trade School Diploma	87	22.14
University Degree	261	66.41
Missing	2	0.51
Household Income ($)		
≤20,000	21	5.34
20,001-40,000	28	7.12
40,001-60,000	39	9.92
60,001-80,000	68	17.30
80,001-100,000	91	23.16

>100,000	136	34.61
Missing	10	2.54
Mother's Marital Status		
Married	275	69.97
Separated	2	0.51
Divorced	1	0.25
Single	15	3.82
Other/Unknown	100	25.45
Mother's Country of Birth		
Canada	337	85.75
Other	56	14.25
Mother's Race		
White	351	89.31
Other	42	10.69
Mother's Smoking Status (Active)		
Prior to pregnancy	113	28.75
Occasionally during pregnancy	8	2.04
Daily during pregnancy	11	2.80
Never	261	66.41
Mother's Exposure to Environmental Tobacco Smoke (Passive)		
Yes	185	47.07
No	208	52.93
Pre-pregnancy Body Mass Index (kg/m^2)		
≤18.50	10	2.54
18.50-24.99	212	53.94
25.00-29.99	83	21.12
>30	61	15.52
Missing	27	6.87
Season of Urine Collection		
Spring	97	24.68
Summer	94	23.92
Fall	123	31.30
Winter	79	20.10
Time of Urine Collection		
6:00-9:00	9	2.29
9:00-12:00	198	50.38
12:00-15:00	123	31.30
15:00-18:00	60	15.27
18:00-24:00	3	0.76
Time Since Last Urination (min)		
≤75	99	25.19
76-120	111	28.24
121-170	60	15.27
>170	101	25.70
Missing	22	5.60
Gender of Baby		
Male	199	50.64
Female	194	49.36
Mean weight at 6 months (kg)	7.97	
Mean length at 6 months (cm)	68.01	
Mean 2D:4D ratio (left) – male and female	0.94	
Mean 2D:4D ratio (right) – male and female	0.95	

Table 6 reports the overall summary statistics (sample size, minimum, maximum, geometric mean with 95% lower and upper confidence intervals, sample median and

sample 95% percentile) for each contaminant both unadjusted and specific gravity adjusted, detected from urine samples from mothers in the 6-month cohort. The descriptive statistics results were similar to those found in the birth cohort (including the number of contaminants with >50% observations below the LOD).

TABLE 6: SUMMARY OF CONTAMINANTS FOUND IN URINE SAMPLES OF PARTICIPANTS FROM THE MIREC/MIRED-ID STUDY(6-MONTH COHORT)

Contaminant (µg/L)	N	LOD	%<LOD	Min	Max	Geometric Mean	95% Confidence Interval		Sample Median	Sample 95th Percentile
							Lower	Upper		
Specific Gravity	418	.	0.00	1.00	1.04	1.01	1.01	1.01	1.01	1.03
MnBP	393	0.20	0.25	ND	800.00	10.56	9.38	11.88	12.00	65.00
MnBP_adj	393	0.20	0.25	0.45	800.00	12.79	11.80	13.86	12.32	42.55
MBzP	392	0.20	0.51	ND	230.00	5.00	4.41	5.67	5.10	39.00
MBzP_adj	392	0.20	0.51	ND	312.00	6.02	5.45	6.66	5.31	37.44
MCHP	393	0.20	95.17	ND	65.00	ND	ND	ND	ND	ND
MCHP_adj	393	0.20	95.17	ND	30.18	ND	ND	ND	ND	0.65
MCPP	393	0.20	19.85	ND	72.00	0.75	0.64	0.86	0.80	8.90
MCPP_adj	393	0.20	19.85	ND	72.00	0.90	0.80	1.02	0.87	8.67
MEHHP	393	0.40	1.53	ND	520.00	7.46	6.62	8.42	8.50	46.00
MEHHP_adj	393	0.40	1.53	ND	355.79	9.04	8.35	9.78	8.84	32.50
MEHP	390	0.20	1.79	ND	110.00	1.88	1.68	2.10	2.10	9.90
MEHP_adj	390	0.20	1.79	ND	52.96	2.26	2.09	2.44	2.13	9.10
MEOHP	393	0.20	0.76	ND	290.00	5.29	4.72	5.93	6.10	28.00
MEOHP_adj	393	0.20	0.76	0.33	171.05	6.41	5.96	6.89	6.41	21.27
MEP	393	0.50	0.25	ND	13000.00	27.21	23.28	31.81	25.00	420.00
MEP_adj	393	0.50	0.25	0.54	20800.00	32.95	28.58	38.00	27.73	403.00
MMP	393	5.00	83.97	ND	32.00	ND	ND	ND	ND	10.00
MMP_adj	393	5.00	83.97	ND	32.50	ND	ND	ND	ND	11.56
MiNP	393	0.40	98.73	ND	6.20	ND	ND	ND	ND	ND
MiNP_adj	393	0.40	98.73	ND	2.99	ND	ND	ND	ND	0.87
MnOP	393	0.70	97.20	ND	1.00	ND	ND	ND	ND	ND
MnOP_adj	393	0.70	97.20	ND	4.55	ND	ND	ND	ND	1.52
Total BPA	418	0.20	11.72	ND	17.00	0.73	0.65	0.81	0.76	4.80
Total BPA_adj	418	0.20	11.72	ND	21.67	0.84	0.77	0.92	0.81	4.68
Free BPA	417	0.012	60.43	ND	1.24	ND	ND	ND	ND	0.09
Free BPA_adj	417	0.012	60.43	ND	0.61	ND	ND	ND	ND	0.10
Total TCS	417	0.12	1.92	ND	2322.96	12.28	9.82	15.35	8.65	665.07
Total TCS_adj	417	0.12	1.92	ND	2745.32	14.18	11.40	17.63	9.10	598.53
Free TCS	417	0.008	21.10	ND	156.42	0.08	0.06	0.10	0.06	5.31
Free TCS_adj	417	0.008	21.10	ND	92.43	0.09	0.07	0.11	0.07	4.39

4.3 Variations in maternal non-occupational exposure to BPA, TCS and select phthalate metabolites

As illustrated in Figure 5, there was significant variation in the geometric mean urinary concentrations across priority environmental contaminants, including several phthalate monoester metabolites, total and free BPA and total and free TCS amongst a sample of mothers participating in the MIREC/MIREC-ID study. As depicted in the figure, several of the contaminants did not have a high cumulative concentration overall based on the sum of the individual concentrations from the maternal urine samples. Further, several of the contaminant geometric mean concentrations were below the LOD, including the levels of MCHP, MMP, MiNP, MnOP and free BPA. There was a relatively even geometric mean concentration of MBzP and MEOHP in the sample of women. Lastly, there was a significantly large presence of MEP overall; it was found to have geometric mean concentration of 27.21 µg/ L.

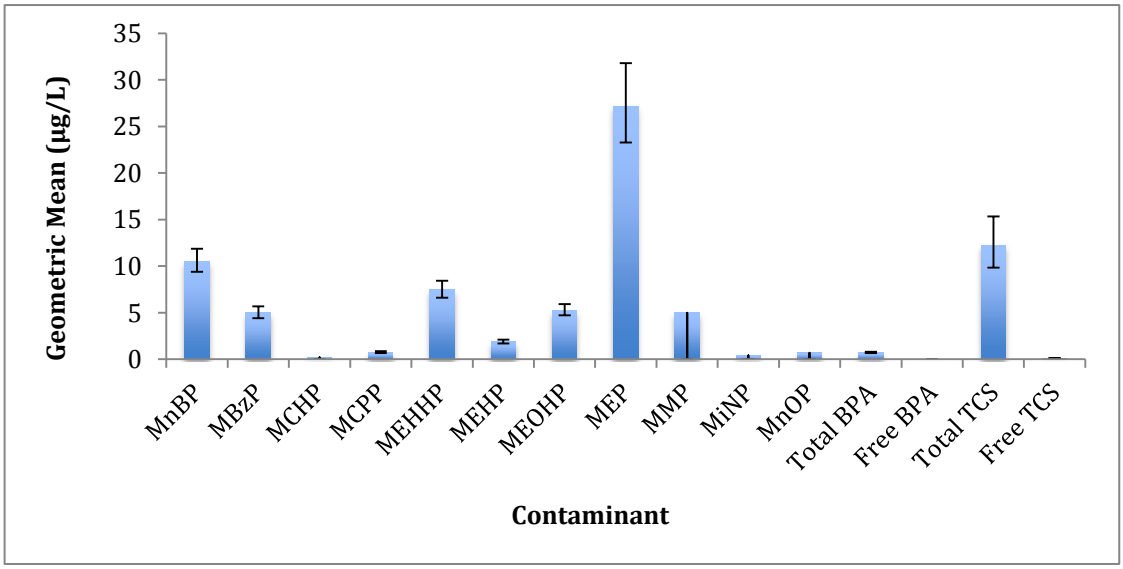


FIGURE 5: MATERNAL NON-OCCUPATIONAL EXPOSURE TO SELECT ENVIRONMENTAL CHEMICALS AMONGST MOTHERS (MEASURED FROM MOTHERS IN THE 6-MONTH COHORT)

As illustrated in Figure 6, the variation in the geometric mean urinary concentration of select phthalate metabolites, total and free BPA and total and free TCS did not vary significantly amongst mothers of White race versus those of other races. These included mothers of Chinese, South Asian, Black, Filipino, Southeast Asian, Latin American, Arab, West Asian, Japanese, Korean and Aboriginal races. This was the case for all contaminants measured, with the exception of MEP. Mothers of White race had a lower concentration of MEP (geometric mean concentration of 25.95 µg/L) as compared to the mothers of other races (geometric mean concentration of 40.46 µg/L). However, this difference was not statistically significant (p=0.08).

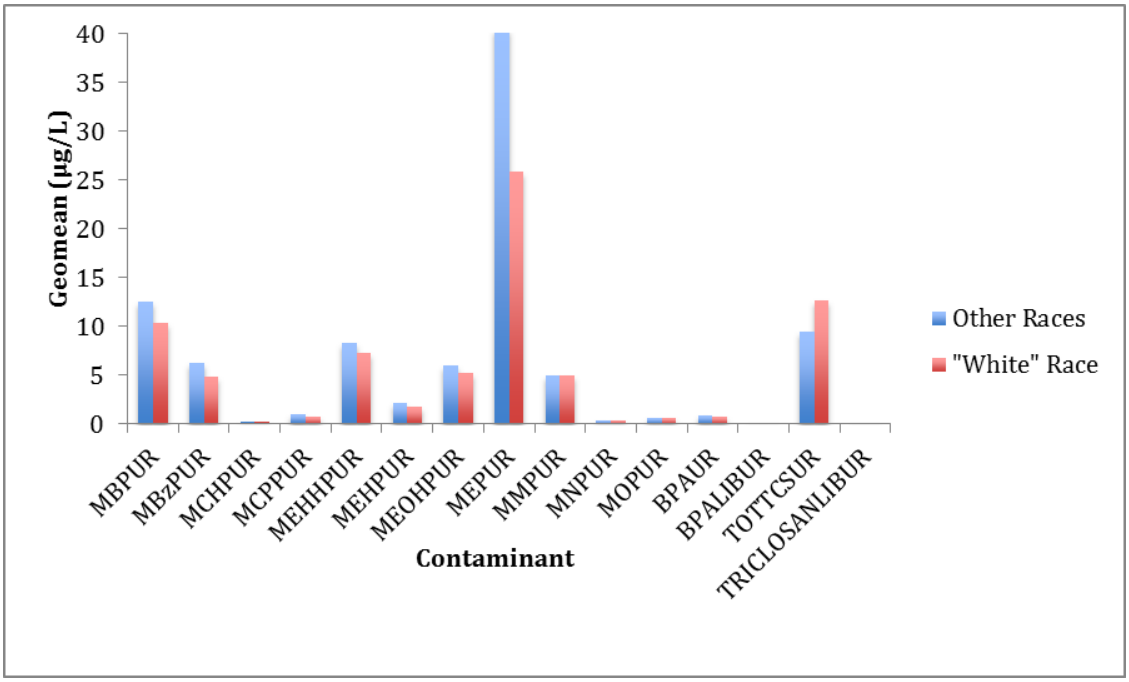


FIGURE 6: VARIATION IN MATERNAL NON-OCCUPATIONAL EXPOSURE TO SELECT ENVIRONMENTAL CHEMICALS AMONGST MOTHERS STRATIFIED BY RACE (MEASURED FROM MOTHERS IN THE 6-MONTH COHORT)

4.4 Evidence supporting each of the research objectives

The biospecimen laboratory data (exposure), outcome and the covariate datasets were merged for both the birth cohort set of maternal-infant pairs, in addition to the 6-month cohort set of maternal-infant pairs.

4.4.1 Objective 1: Association between maternal non-occupational exposure to select environmental contaminants and anogenital distance in newborn infants

Several multiple linear regression models were created to test for the association between various environmental contaminants (phthalate monoester metabolites, BPA, TCS) and the distance between anus and the cephalad base of the penis (anogenital distance) in male infants. The results of the simple linear regression models (i.e. univariate linear regression) for the outcome of AGD can be found in Appendix 4. Several of these results were significant at $p \leq 0.05$.. The multivariable models were controlled for specific gravity, in addition to various other maternal and infant characteristics that may have confounded the relationship between the exposure and the outcome (as confounders are related to both the exposure and outcome and are not on the causal pathway). These were determined on the basis of several independent univariate tests; any variables that had a $p \leq 0.15$ were included in further analyses. These included: maternal education, maternal marital status, maternal race, maternal active smoke exposure, season of urine collection, time of urine collection, time since last urine void, infant weight for length z score, infant birth weight and infant birth length. Upon fitting the best model, final models included the following covariate variables: specific gravity, maternal race and infant birth weight. Table 7 below reports the results of these models. None of the models found significant predictors of the anogenital distance in

male infants; thus the null hypothesis cannot be rejected for any of the models at p≤0.05. The models for the monoester metabolites MEOHP and MEP almost reached statistical significance (p=0.057 and p=0.085, respectively).

TABLE 7: REGRESSION ANALYSES OF LOG MONOESTER METABOLITE AND SUM LOW AND HIGH MOLECULAR WEIGHT PHTHALATE CONCENTRATIONS ON ANOGENITAL DISTANCE IN MALE INFANTS, CONTROLLING FOR SELECT MATERNAL AND INFANT COVARIATE VARIABLES (P≤0.05)

	Anogenital Distance (male infants)[a]	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	0.508 (-.355, 1.370)	0.250
MBzP	0.016 (-.656, 0.688)	0.963
MCPP	-.314 (-.912, 0.283)	0.304
MEHHP	0.530 (-.301, 1.362)	0.213
MEHP	0.618 (-.256, 1.493)	0.168
MEOHP	0.859 (-.020, 1.738)	0.057
MEP	0.428 (-.057, 0.914)	0.085
TOTBPA	-.324 (-1.04, 0.394)	0.377
TOTTCS	0.026 (-.284, 0.336)	0.871
TRICLOSANLIB	-.035 (-.348, 0.278)	0.828
LMW_Pthalates[b]	0.535 (-.171, 1.241)	0.139
HMW_Phthalates[c]	0.686 (-.254, 1.627)	0.155

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate; TOTBPA=total bisphenol a; TOTTCS=total triclosan; TRICLOSANLIB=free triclosan; CI=confidence interval

[a] Controlled for: specific gravity, maternal race and infant birth weight.

[b] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[c] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

Additionally, several multiple linear regression models were created to test for the association between various environmental contaminants (phthalate monoester metabolites, BPA, TCS) and the distance between anus and the clitoris (anus-clitoris distance) in female infants. Based on the results of several independent univariate tests, the following variables were included for further analyses (p≤0.15): maternal age, maternal education, maternal marital status, household income, mother's birth country, time of urine collection, time since last urine void, infant weight for length z score, and infant birth weight. Upon fitting the best model, final models included the following covariate variables: specific gravity and infant birth weight. Table 8 below reports the results of these models. Two of the models are significant predictors of the anus-clitoris distance in female infants at p≤0.05. Changes in the

concentrations of the monoester metabolites MBzP and MEP are related to changes in the distance between the anus-clitoris in female infants. As reported, a one-unit increase in the concentration of MBzP will result in a .901 mm decrease in the distance between the anus and clitoris (p=0.002). Moreover, a one-unit increase in the concentration of MEP results in a 0.579 mm increase in the distance between the anus and clitoris (p=0.008). The model for MnBP and the sum of all the high molecular weight phthalate monoester metabolites almost reached statistical significance (p=0.066 and p=0.078, respectively).

TABLE 8: REGRESSION ANALYSES OF LOG MONOESTER METABOLITE AND SUM LOW AND HIGH MOLECULAR WEIGHT PHTHALATE CONCENTRATIONS ON ANUS-CLITORIS DISTANCE IN FEMALE INFANTS, CONTROLLING FOR SELECT MATERNAL AND INFANT COVARIATE VARIABLES (P≤0.05)

	Anus-clitoris Distance (female infants)[a]	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	-.640 (-1.32, 0.037)	0.066
MBzP	-.901 (-1.46, -.345)	0.002
MCPP	-.386 (-.923, 0.151)	0.161
MEHHP	-.578 (-1.29, 0.137)	0.115
MEHP	-.501 (-1.27, 0.270)	0.205
MEOHP	-.541 (-1.30, 0.218)	0.164
MEP	0.579 (0.159, 0.999)	0.008
TOTBPA	-.341 (-.929, 0.247)	0.257
TOTTCS	0.001 (-.006, 0.009)	0.724
TRICLOSANLIB	-.002 (-.010, 0.007)	0.724
LMW_Pthalates[b]	0.496 (-.097, 1.089)	0.103
HMW_Phthalates[c]	-.764 (-1.61, 0.081)	0.078

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate; TOTBPA=total bisphenol a; TOTTCS=total triclosan; TRICLOSANLIB=free triclosan; CI=confidence interval

[a] Controlled for: specific gravity and infant birth weight.

[b] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[c] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

Furthermore, several multiple linear regression models were created to test for the association between various environmental contaminants (phthalate monoester metabolites, BPA, TCS) and the distance between anus and the scrotum (anoscrotal distance) in male infants. Based on the results of several independent univariate tests, the following variables were included for further analyses (p≤0.15): maternal BMI, maternal marital status, maternal passive smoke exposure, season of urine collection, infant gestational age, infant birth

weight, and infant birth length. Upon fitting the best model, final models included the following covariate variables: specific gravity and infant birth weight. Table 9 below reports the results of these models. None of the models are significant predictors of the anoscrotal distance in male infants; thus the null hypothesis cannot be rejected for any of the models at $p \leq 0.05$.

TABLE 9: REGRESSION ANALYSES OF LOG MONOESTER METABOLITE AND SUM LOW AND HIGH MOLECULAR WEIGHT PHTHALATE CONCENTRATIONS ON ANOSCROTAL DISTANCE IN MALE INFANTS, CONTROLLING FOR SELECT MATERNAL AND INFANT COVARIATE VARIABLES (P≤0.05)

	Anoscrotal distance (male infants)[a]	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	-.359 (-1.20, 0.486)	0.406
MBzP	-.122 (-.785, 0.542)	0.720
MCPP	-.246 (-.840, 0.348)	0.419
MEHHP	0.333 (-.489, 1.155)	0.428
MEHP	0.296 (-.560, 1.151)	0.499
MEOHP	0.363 (-.512, 1.238)	0.417
MEP	-.102 (-.585, 0.380)	0.678
TOTBPA	0.479 (-.187, 1.145)	0.160
TOTTCS	-.048 (-.340, 0.244)	0.749
TRICLOSANLIB	0.044 (-.251, 0.339)	0.772
LMW_Pthalates[b]	-.388 (-1.08, 0.307)	0.275
HMW_Phthalates[c]	0.381 (-.547, 1.309)	0.422

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate; TOTBPA=total bisphenol a; TOTTCS=total triclosan; TRICLOSANLIB=free triclosan; CI=confidence interval

[a] Controlled for: specific gravity and infant birth weight.

[b] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[c] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

Lastly, several multiple linear regression models were created to test for the association between various environmental contaminants (phthalate monoester metabolites, BPA, TCS) and the distance between anus and the fourchette (anus-fourchette distance) in female infants. Based on the results of several independent univariate tests, the following variables were included for further analyses ($p \leq 0.15$): maternal education, maternal active smoke exposure, maternal passive smoke exposure, time since last urine void and infant weight by length z score. Upon fitting the best model, final models included only specific gravity as a covariate variable. Table 10 below reports the results of these models. None of

the models are significant predictors of the anus-fourchette distance in female infants; thus the null hypothesis cannot be rejected for any of the models at p≤0.05. The model for the monoester metabolite MBzP almost reached statistical significance (p=0.070).

TABLE 10: REGRESSION ANALYSES OF LOG MONOESTER METABOLITE AND SUM LOW AND HIGH MOLECULAR WEIGHT PHTHALATE CONCENTRATIONS ON ANUS-FOURCHETTE DISTANCE IN FEMALE INFANTS, CONTROLLING FOR SELECT MATERNAL AND INFANT COVARIATE VARIABLES (P≤0.05)

	Anus-fourchette distance (female infants)[a]	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	0.009 (-.036, 0.053)	0.704
MBzP	-.034 (-.070, 0.003)	0.070
MCPP	0.004 (-.031, 0.039)	0.825
MEHHP	-.002 (-.049, 0.044)	0.917
MEHP	0.020 (-.030, 0.070)	0.432
MEOHP	-.006 (-.055, 0.043)	0.810
MEP	0.005 (-.023, 0.033)	0.728
TOTBPA	-.001 (-.040, 0.037)	0.951
TOTTCS	-0.003 (-.021, 0.014)	0.695
TRICLOSANLIB	0.002 (-.018, 0.021)	0.879
LMW_Pthalates[b]	0.011 (-.028, 0.050)	0.576
HMW_Phthalates[c]	-.003 (-.058, 0.052)	0.925

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate; TOTBPA=total bisphenol a; TOTTCS=total triclosan; TRICLOSANLIB=free triclosan; CI=confidence interval

[a] Controlled for: specific gravity.

[b] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[c] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

4.4.2 Objective 2: Association between maternal non-occupational exposure to select environmental contaminants and 2D:4D finger digit ratio in 6-month old infants

The results of the simple linear regression models (i.e. univariate linear regression) for the outcome of 2D:4D digit ratio can also be found in Appendix 4. Several of these results were significant at p≤0.05. Following this, multiple linear regression models were created to test for the association between various environmental contaminants (phthalate monoester metabolites, BPA, TCS) and the 2D:4D digit ratio in the left hand of male infants. The models were controlled for specific gravity, in addition to various other maternal and infant characteristics that may have confounded the relationship between the exposure and the

outcome. These were determined on the basis of several independent univariate tests; any variables that had a p≤0.15 were included in further analyses. These included: maternal education, maternal birth country, time since last urine void, and 6-month infant length. Upon fitting the best model, final models included the following covariate variables: specific gravity and 6-month infant length. Table 11 below reports the results of these models. One model is a significant predictor of the 2D:4D digit ratio in the left hand of male infants at p≤0.05. Changes in the concentrations of the monoester metabolite MEHHP is related to changes in the 2D:4D digit ratio in the left hand of male infants. As reported, a one-unit increase in the concentration of MEHHP results in a .010 times decrease in the digit ratio (p=0.021). The models for MEHP and TRICLOSANLIB almost reached statistical significance (p=0.092 and p=0.098, respectively).

TABLE 11: REGRESSION ANALYSES OF LOGE MONOESTER METABOLITE AND SUM LOW AND HIGH MOLECULAR WEIGHT PHTHALATE CONCENTRATIONS ON 2D:4D DIGIT RATIO IN LEFT HANDS IN MALE INFANTS, CONTROLLING FOR SELECT MATERNAL AND INFANT COVARIATE VARIABLES (P≤0.05)

	2D:4D digit ratio (left hand, male infants)[a]	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	-.001 (-.010, 0.008)	0.845
MBzP	-.001 (-.006, 0.004)	0.646
MCPP	-.001 (-.007, 0.005)	0.660
MEHHP	-.010 (-.019, -.002)	0.021
MEHP	-.008 (-.017, 0.001)	0.092
MEOHP	-.008 (-.018, 0.002)	0.115
MEP	0.001 (-.004, 0.007)	0.632
TOTBPA	-.005 (-.012, 0.003)	0.260
TOTTCS	-.003 (-.006, 0.001)	0.135
TRICLOSANLIB	-.003 (-.006, 0.001)	0.098
LMW_Pthalates[b]	-.002 (-.010, 0.007)	0.727
HMW_Phthalates[c]	-.001 (-.011, 0.009)	0.863

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate; TOTBPA=total bisphenol a; TOTTCS=total triclosan; TRICLOSANLIB=free triclosan; CI=confidence interval

[a] Controlled for: specific gravity, and 6-month infant length.

[b] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[c] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

Additionally, several multiple linear regression models were created to test for the association between various environmental contaminants (phthalate monoester metabolites,

BPA, TCS) and the 2D:4D digit ratio in the left hand of female infants. Based on the results of several independent univariate tests, the following variables were included for further analyses ($p \leq 0.15$): maternal education, maternal marital status, household income, season of urine collection, 6-month infant weight, and 6-month infant length. Upon fitting the best model, final models only specific gravity as a covariate variable. Table 12 below reports the results of these models. None of the models are significant predictors of the 2D:4D digit ratio in left hand of female infants; thus the null hypothesis cannot be rejected for any of the models at $p \leq 0.05$.

TABLE 12: REGRESSION ANALYSES OF LOGE MONOESTER METABOLITE AND SUM LOW AND HIGH MOLECULAR WEIGHT PHTHALATE CONCENTRATIONS ON 2D:4D DIGIT RATIO IN LEFT HANDS IN FEMALE INFANTS, CONTROLLING FOR SELECT MATERNAL AND INFANT COVARIATE VARIABLES (P≤0.05)

	2D:4D digit ratio (left hand, female infants)[a]	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	0.001 (-.008, 0.010)	0.814
MBzP	-.001 (-.006, 0.004)	0.668
MCPP	-.001 (-.009, 0.006)	0.692
MEHHP	-.002 (-.012, 0.009)	0.756
MEHP	-.003 (-.014, 0.008)	0.600
MEOHP	-.004 (-.016, 0.007)	0.464
MEP	-.001 (-.006, 0.004)	0.668
TOTBPA	-.002 (-.010, 0.006)	0.560
TOTTCS	0.001 (-.002, 0.004)	0.565
TRICLOSANLIB	-.000 (-.004, 0.003)	0.954
LMW_Pthalates[b]	-.001 (-.009, 0.008)	0.899
HMW_Phthalates[c]	0.000 (-.012, 0.013)	0.972

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate; TOTBPA=total bisphenol a; TOTTCS=total triclosan; TRICLOSANLIB=free triclosan; CI=confidence interval

[a] Controlled for: specific gravity.

[b] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[c] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

Furthermore, several multiple linear regression models were created to test for the association between various environmental contaminants (those above the LOD) and the 2D:4D digit ratio in the right hand of male infants. Based on the results of several independent univariate tests, the following variables were included for further analyses ($p \leq 0.15$): maternal age, maternal BMI, maternal birth country, and season of urine collection.

Upon fitting the best model, final models only included specific gravity as a covariate variable. Table 13 below reports the results of these models. One model was a significant predictor of the 2D:4D digit ratio in the right hand of male infants at $p \leq 0.05$. A change in the concentrations of TOTBPA is related to changes in the 2D:4D digit ratio in the right hand of male infants. As reported, a one-unit increase in the concentration of TOTBPA results in a .012 times decrease in the digit ratio (p=0.002).

TABLE 13: REGRESSION ANALYSES OF LOGE MONOESTER METABOLITE AND SUM LOW AND HIGH MOLECULAR WEIGHT PHTHALATE CONCENTRATIONS ON 2D:4D DIGIT RATIO IN RIGHT HANDS IN MALE INFANTS, CONTROLLING FOR SELECT MATERNAL AND INFANT COVARIATE VARIABLES (P≤0.05)

	2D: 4D digit ratio (right hand, male infants)[a]	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	0.004 (-.005, 0.013)	0.381
MBzP	0.002 (-.004, 0.007)	0.509
MCPP	-.003 (-.009, 0.003)	0.368
MEHHP	-.002 (-.011, 0.007)	0.676
MEHP	-.003 (-.012, 0.006)	0.561
MEOHP	-.002 (-.012, 0.008)	0.714
MEP	-.001 (-.006, 0.004)	0.657
TOTBPA	-.012 **(-.020, -.004)**	**0.002**
TOTTCS	-.001 (-.004, 0.003)	0.730
TRICLOSANLIB	-.001 (-.004, 0.003)	0.738
LMW_Pthalates[b]	-.001 (-.009, 0.008)	0.862
HMW_Phthalates[c]	-.003 (-.013, 0.008)	0.634

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate; TOTBPA=total bisphenol a; TOTTCS=total triclosan; TRICLOSANLIB=free triclosan; CI=confidence interval

[a] Controlled for: specific gravity.

[b] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[c] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

Lastly, several multiple linear regression models were created to test for the association between various environmental contaminants (phthalate monoester metabolites, BPA, TCS) and the 2D:4D digit ratio in the right hand of female infants. Based on the results of several independent univariate tests, the following variables were included for further analyses ($p \leq 0.15$): maternal education, maternal passive smoke exposure, maternal active smoke exposure, season of urine collection, and time of urine collection. Upon fitting the best model, final models only included specific gravity as a covariate variable. Table 14

below reports the results of these models. One model is a significant predictor of the 2D:4D digit ratio in the right hand of female infants at p≤0.05. A change in the concentrations of MBzP is related to changes in the 2D:4D digit ratio in the right hand of female infants. As reported, a one-unit increase in the concentration of MBzP results in a .006 times increase in the digit ratio (p=0.024).

TABLE 14: REGRESSION ANALYSES OF LOGE MONOESTER METABOLITE AND SUM LOW AND HIGH MOLECULAR WEIGHT PHTHALATE CONCENTRATIONS ON 2D:4D DIGIT RATIO IN RIGHT HANDS IN FEMALE INFANTS, CONTROLLING FOR SELECT MATERNAL AND INFANT COVARIATE VARIABLES (P≤0.05)

	2D: 4D digit ratio (right hand, female infants)[a]	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	-.002 (-.012, 0.008)	0.702
MBzP	0.006 **(0.001, 0.011)**	**0.024**
MCPP	-.000 (-.008, 0.007)	0.972
MEHHP	0.003 (-.008, 0.013)	0.644
MEHP	0.009 (-.002, 0.021)	0.101
MEOHP	0.005 (-.007, 0.017)	0.411
MEP	-.001 (-.006, 0.004)	0.720
TOTBPA	0.002 (-.006, 0.010)	0.639
TOTTCS	0.001 (-.002, 0.004)	0.499
TRICLOSANLIB	0.000 (-.003, 0.004)	0.807
LMW_Pthalates[b]	0.004 (-.005, 0.013)	0.363
HMW_Phthalates[c]	0.002 (-.010, 0.015)	0.736

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate; TOTBPA=total bisphenol a; TOTTCS=total triclosan; TRICLOSANLIB=free triclosan; CI=confidence interval

[a] Controlled for: specific gravity.

[b] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[c] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

4.4.3 Objective 3: Correlation between the anogenital distance and the 2D:4D digit ratio

As reported in Table 15, the Spearman's rank correlation coefficients (r) between the short and long anogenital distance and the 2D:4D digit ratio in both the left and right hands are all close to 0, indicating the variables are independent of each other (i.e. no dependence).

TABLE 15: SPEARMAN'S RANK CORRELATION COEFFICIENT TO TEST DEPENDENCE BETWEEN LONG AND SHORT ANOGENITAL DISTANCE AND THE LEFT AND RIGHT HAND 2D:4D DIGIT RATIO

	Spearman's rank correlation coefficient (*P* value)			
	Long anogenital distance measure[a] (# of observations)	**Short anogenital distance measure[b] (# of observations)**	**2D:4D digit ratio (left hand) (# of observations)**	**2D:4D: digit ratio (right hand) (# of observations)**

Long anogenital distance measure[a]	1.00000 (312)	0.66374 (<0.0001) (310)	0.08709 (0.1260) (310)	0.04475 (0.4309) (312)
Short anogenital distance measure[b]	-	1.00000 (314)	0.10684 (0.0594) (312)	0.09180 (0.1045) (314)
2D:4D digit ratio (left hand)	-	-	1.00000	0.55930 (<0.0001) (314)
2D:4D: digit ratio (right hand)	-	-	-	1.00000 (316)

[a] Anus-cephalad base of the penis distance in male infants; anus-clitoris distance in female infants
[b] Anoscrotal distance in male infants; anus-fourchette distance in female infants

CHAPTER 5: DISCUSSION

This prospective cohort study is the first Canadian study to examine associations between prenatal exposures to common environmental chemicals and reproductive sensitive endpoint measures in newborn infants.

5.1 Explanation of study findings

5.1.1 Descriptive Characteristics

The MIREC cohort study recruited pregnant women across multiple clinical sites in Canada. While the study attempted to recruit patients of varying education level and socioeconomic status, the sample population employed in this study was highly educated, where 61% of participants had a university degree (in the birth cohort). When compared to the largest AGD study to date in a human population, this number was slightly smaller than the percentage of participants who had graduated college (74.36%) (76). Further, over 50% of participants had an overall household annual income of greater than $80,000 in the birth cohort (i.e. 21.89% of the sample had an income between $80,001-100,000 and 30.54% of the sample had an income greater than $100,000). As determined Barrett et al. (102), women with a higher education level (and potentially income) are more likely to be aware of the dangers of environmental chemicals which in turn is often related to them exhibiting more healthy behaviours, such as choosing chemical-free personal care products. In a study conducted by Arbuckle et al. (103), in the overall large sample of approximately 2000 women who participated in the MIREC study, it was determined that the geometric mean concentration of BPA was higher in women who had a lower income or were less educated.

As the sample of participants for this study was taken from this large population, there may be a differential exposure to chemicals compared between participants who were less educated versus more educated. Other characteristics of note were country of birth and time of urine collection. Most participants were born in Canada (81% of the sample from the birth cohort) and had their urine collected during 09:00 AM and 12:00 PM. Free TCS was found to vary by time of urine collection – women with urine collected during 09:00 AM and 12:00 PM had higher concentrations than those with urine collected during 03:00 and 06:00 PM (103). Overall, the descriptive characteristic observed in the sample of mothers in this study was similar to that seen in the largest AGD study to date, with the only large difference seen in race (76). In this study, 90% of the participants were white; on the other hand, in the study by Swan et al. (76) only 66% were white. It should be noted that, as expected, the descriptive characteristics of the 6-month cohort sample of women were similar to that of the birth cohort, as most participants (mother-infant pairs) completed both the birth and the 6-month assessments.

The sample population had a relatively even number of female and male infants. The average infant birth weight (3.34 kg) was similar to that observed in the study by Swan et al. (76) (3.37 kg). The average birth weight is under the 50^{th} percentile and the average infant birth length (50.47 cm) fell under the 85^{th} percentile, based on the World Health Organization growth charts standards for Canada (94).

The summary statistics for each of the contaminants detected in maternal urine samples indicated a few issues of note. Firstly, as mentioned, several of the phthalate metabolites were not prevalent in this sample of participants (MCHP, MMP, MiNP, MnOP); over 50% of urine samples obtained for these contaminants had measured concentrations

below the laboratory's LOD. The phthalate metabolites with the highest geometric mean (GM) concentrations were determined to be: MEP (specific gravity (SG)-adjusted GM of 32.92 μg/L; detected in >99% of samples) and MnBP (SG-adjusted GM 12.92 μg/L; detected in 100% of samples). These results were similar to the study conducted by Swan et al. (76), where the phthalate metabolite with the highest geometric mean concentration was also MEP (28.4 ng/mL) (see Table 16). Secondly, for the BPA urinary contaminant, total BPA (free plus conjugated forms) had a SG-adjusted GM of 0.90 μg/L, with it being detected in approximately 88% of samples. On the other hand, the overall geometric mean urinary concentration of free BPA was not prevalent in the population; this is expected as many studies have shown relatively low levels of free BPA in blood and urine in humans due to the body's relatively fast metabolism of BPA – with an elimination half-life of approximately 6 hours (104–107). Lastly, total TCS had a SG-adjusted GM urinary concentration of 11.92 μg/L (detected in approximately 98% of samples), while free TCS had an SG-adjusted GM urinary concentration of 0.08 μg/L (detected in approximately 77% of samples). All of the aforementioned results were similar between the birth and 6-month cohorts. Additionally, the results of these summary statistics are in accordance with the overall concentrations of urinary contaminants reported in the overall MIREC study population (88)

When comparing the geometric mean concentrations of the various phthalate metabolites from first trimester urine samples from this study (MIREC/MIREC-ID participants) with that of the study by Swan et al. (76) (TIDES) as seen in Table 16, it can be seen that overall, the concentrations reported in the study by Swan et al. (76) were smaller than those reported in this study, with the exception of the phthalate metabolite MCPP, which had a higher geometric mean concentration in the study by Swan et al. (76).

TABLE 16: COMPARISON OF THE GEOMETRIC MEAN CONCENTRATIONS OF PHTHALATE METABOLITES FROM FIRST TRIMESTER URINE SAMPLES – MIREC/MIREC-ID VS. TIDES (*IN ITALICS*)

Contaminant (µg/L)	Geometric Mean	95% Confidence Interval	
		Lower	Upper
MnBP	12.92 *6.36*	11.92 *5.77*	14.02 *7.00*
MBzP	6.20 *3.31*	5.59 *2.98*	6.88 *3.68*
MCPP	0.88 *1.91*	0.79 *1.72*	0.99 *2.13*
MEHHP	9.23 *6.04*	8.51 *5.49*	10.02 *6.64*
MEHP	2.33 *1.93*	2.15 *1.76*	2.53 *2.11*
MEOHP	6.54 *4.22*	6.06 *3.84*	7.05 *4.63*
MEP	32.92 *28.4*	28.64 *25.3*	37.83 *31.9*

The results suggest there is substantial variation in the presence of common environmental chemicals amongst pregnant women in Canada, as detected in maternal urine samples. As reported in the study by Arbuckle et al. (88), the concentrations of the metabolites measured is comparable to that observed in women between the ages of 20-39 years in cycle 2 of the Canadian Health Measures Survey (CHMS) (43), but lower than what was reported in an American and Spanish pregnancy cohort (108,109). Furthermore, the median concentration of MEP observed in the MIREC cohort study was substantially higher than the American and Spanish cohorts. Explanation for these differences include differences in the time period when data was collected (and thus there may be a decline in the overall availability of phthalate containing products in response to risk assessment measures) in addition to differences in the study populations, where participants in the American cohort were overall of lower socioeconomic status (88). In regards to the BPA levels measured, as reported in the study by Arbuckle et al. (103), the total BPA measured in this study is relatively lower than what has been reported in several cohort pregnancy studies in the

United States. A study that compared the differences in urinary BPA concentrations between Canadian and US populations found no significant differences in the methodology employed (110), suggesting other differences. Differences between urinary concentrations of BPA and TCS measured in the MIREC study versus urinary concentration measured in other national studies in other countries, may be due to differences in laboratory methods, study populations, hydrolysis of the conjugates or contamination of the study samples (103).

5.1.2 Phthalate metabolites: predictors of the AGD or 2D:4D digit ratio in female and/or male infants?

The findings from this study indicate that in female infants, the monoester phthalate metabolite MBzP (a metabolite of parent phthalate BBzP) was significantly associated with a reduced anus-clitoris distance (ß=-.901; p=0.002), while the metabolite MEP (a metabolite of parent phthalate DEP) was significantly associated with a larger anus-clitoris distance (ß=0.579; p=0.008). As the coefficients are logarithmically transformed to account for non-normalized distributions, the results indicate that a 1% increase in the concentration MBzP will result in a 0.00901 mm decrease in the anus-clitoris distance and a 1% in the concentration of MEP will result in a 0.00579 mm increase in the anus-clitoris distance. To date, the only health effects that have been studied to be associated with increased exposure to MBzP and MEP phthalate metabolites in infants found an increased risk of ADHD-like and autistic-like behaviours, increased BMI and waist circumference (in females only), a higher likelihood of internalizing behaviours and an increased risk of allergic diseases (39).

No monoester phthalate metabolites were significantly associated with predicting the anogenital or the anoscrotal distance in male infants (p>0.05). The monoester metabolite MEOHP may be a significant predictor of AGD in male infants, but it only reached

borderline significance (ß=0.859; p=0.057). The results of this study are not entirely consistent with that of previous studies conducted in male infants (13,14,41,73,74,76), which found significant associations between mainly DEHP metabolites and reduced AGD in male infants. This is assumed to be via a reduction in testosterone production. However, results of this study are consistent with findings from one study conducted in Taiwanese infants (75), which found no significant association between prenatal phthalate exposure and anogenital index in male infants. This study concluded that prenatal exposure to phthalates might not be affecting testosterone level of male fetuses at the exposure level measured in the Taiwanese population (75). This is supported by several in-vitro studies that found no association between select phthalate metabolite exposure and fetal testosterone synthesis (111,112) – which may be also be suggesting that phthalates act via direct effect on the reproductive tract instead of via testosterone levels. The lack of consistency with previous studies may be due to a number of reasons. There may have been a differential exposure to phthalate containing products due to an increased public awareness and concern around the use of phthalate containing products, resulting in smaller geometric mean metabolite concentrations in measured maternal urine. The concentrations of several phthalate metabolites in the recent study by Bornehag et al. (73) were significantly higher than the concentrations in this study, suggesting that potentially, Canadians are more aware of the possible effects of phthalates, and in turn, are limiting their exposure. The differences may also be due to a number of other inherent limitations with this study (or other studies) and may also be due to publication bias, both which will be discussed in a subsequent section.

The monoester phthalate metabolite MEHHP was significantly associated with a reduced 2D:4D digit ratio in the left hand of male infants (ß=-.010; p=0.021); that is, a 1%

increase in the concentration of MEHHP is associated with a .00010 reduced 2D:4D digit ratio in the left hand of male infants. This suggests that the exposure to the MEHHP metabolite is resulting in a normal development of the 2D:4D digit ratio, based on its sexually dimorphic nature. Furthermore, the monoester metabolite MBzP was also significantly associated with a larger 2D:4D digit ratio in the right hand of female infants (ß=0.006; p=0.024); that is, a 1% increase in the concentration of MBzP is associated with a .00006 increased 2D:4D digit ratio in the right hand of female infants. Females are known to have a larger 2D:4D digit ratio (assumed to be cause by differential androgen exposure); if the phthalate metabolite MBzP has anti-androgenic properties (less masculinization), this would then result in a larger ratio.

5.1.3 BPA: predictor of the AGD or 2D:4D digit ratio in female and/or male infants?

No significant associations between BPA exposure and AGD were found in female or male infants. As mentioned by Swan (15), much of the research in the area of the AGD has focused on male rodents and infants; further data is needed around the adverse effects of endocrine disrupting chemicals and reproductive biomarkers in female infants. In regards to the results seen in males, this contrasts with one study, which found that increasing maternal BPA exposure levels in pregnancy (as measured from air samples) were associated with a higher likelihood of shortened AGD in male offspring (77). There is a significant deal of speculation into whether the post-metabolism conjugated form of BPA is harmful as studies have shown that this form of BPA does not display estrogenic activity (107,113).Thus, results of biomonitoring studies that have shown the adverse effects of BPA on various physiological systems are likely due to exposure to unconjugated or ‘free’ form of BPA

(114–116). However, this is also a point of uncertainty as the rapid metabolism of BPA leaves little free BPA in the body (107), as was demonstrated in this study where approximately 60% of the total observations of free BPA were determined to be below the LOD.

Moreover, the results indicated that total BPA was significantly associated with a reduced 2D:4D digit ratio in the right hand of male infants (ß=-.012; p=0.002). That is, a 1% increase in the concentration of total BPA will result in .0012 decrease in the 2D:4D digit ratio in the right hand of male infants. Though there have been no studies that have looked at the association of BPA on 2D:4D digit ratio in infants; again, this result is not expected as BPA has been shown to have anti-androgenic/estrogenic properties

5.1.4 TCS: predictor of the AGD or 2D:4D digit ratio in female and/or male infants?

Finally, results of this study indicate that TCS was not significantly associated with predicting the AGD or the 2D:4D digit ratio in male or female infants. This is the first study to look at the link between TCS and reproductive sensitive endpoint markers, across all species, including humans. Studies conducted in rats have shown TCS to have both estrogenic and androgenic activity, where TCS has shown to bind to both types of receptors in-vitro (72,117). Further, exposure to TCS has been shown to lower levels of the thyroid hormone and testosterone in male rats (71,117). Though these studies indicate the potential adverse effects of TCS in rats, it is difficult to extrapolate these findings to humans as the level of TCS exposure in these studies with rats was substantially higher than one would expect in the normal human environment. Thus, there is a significant lack of evidence around

the potential effects of human exposure to ‘everyday’ levels of TCS, especially at the reproductive level.

5.1.5 General discussion of the study findings

Though these results question the anti-androgenic and/or estrogenic properties of phthalates, BPA and TCS, speculation should center on the utility of the endocrine sensitive markers of exposure – specifically the 2D:4D digit ratio as a sensitive marker of fetal androgen exposure. Sexual differences in the 2D:4D ratio may be due to postnatal factors, between the time of birth and when it is measured in infants, rather than differential androgen exposure (90). Studies in both mice and monkeys show that androgens modulate fetal digit growth (44,118), but not in way that the 2D:4D digit ratio accurately suggests fetal androgen exposure by itself. Further, this is apparent in the lack of correlation between the AGD and 2D:4D observed in this study ($r<1$), in addition to other studies (119,120). Thus, based on the available evidence at this time, AGD is likely a more accurate biomarker of fetal androgen exposure. However, it should also be noted that both the AGD and 2D:4D digit ratio are relatively new markers used in human reproductive toxicity studies. There is a definitive need for more studies to develop and better understand their relevance. Furthermore, it would be interesting to see how these endpoints change throughout adolescence and adulthood, in order to help determine if development at birth or at 6-months may be predictive of other adverse reproductive health outcomes in later years of life.

The findings from this study largely suggest no association between phthalates, BPA, TCS and endpoint measures AGD and 2D:4D, with the exception of a few significant results. When looking at all of the AGD studies conducted in human populations to date, as

presented in Table 1, significant findings were found between select phthalate metabolites and AGD. However, more importantly, there were a higher number of non-significant findings between other measured phthalate metabolites and AGD. When these studies are mentioned in literature, the focus tends to be on the one or two significant findings, mostly ignoring the presence of large insignificant findings. Further, this issue also highlights the potential publication bias in this area of research – where either the investigators or journals (in some cases) may not been keen on publishing insignificant results as they differ from what the body of evidence already says. Perhaps there have been studies which found no such association between maternal phthalate, BPA and/or TCS exposure and AGD and 2D:4D digit ratio in infants, but have never been published.

Further, the select significant associations seen in this study may have simply been due to type I error – where the null hypothesis was rejected but may actually be true. This is the notion of accepting the alternative hypothesis when the results may actually be attributed to chance (and thus, are considered false positive). In statistics, the likelihood of Type I error increases when conducting multiple comparisons and using statistical tests repeatedly. In this analysis, 96 comparisons were conducted in total, suggesting a high likelihood for the occurrence of Type I error. It would be interesting to see if reducing the number of comparisons made or adjusting for multiple comparisons made (i.e. Bonferroni correction) alter the conclusions of this study.

In regards to dealing with values below the LOD for several of the contaminants included in the analysis – although the results would have likely not differed much due to the small percentage of values below the LOD, potentially using a different approach, such as a censoring method would have led to a less biased and more precise estimate (i.e. smaller

mean squared error). Nonetheless, it would be interesting to compare the results of this study using different approaches to dealing with values below the LOD.

5.2 Limitations of this study

The nature of the MIREC study design called for many inherent limitations that should be noted.

This study was likely limited by selection bias. Enrolment for the MIREC study was entirely a voluntary process; and as commonly known, volunteers tend to be more health conscious. Moreover, as mentioned earlier in the discussion, there were large differences observed in the distribution of participants amongst several population level characteristics (i.e. mothers were more educated, had a higher household income). Thus, a combination of these factors may have meant that the mothers enrolled in MIREC were already avoiding exposure to a variety of potentially adverse environmental chemicals. Although the MIREC cohort study recruited pregnant women across multiple clinical sites in Canada so as to obtain a varied and representative sample, as a result of the aforementioned, the mothers in this study may not represent the general population at large, threatening the generalizability – and thus, external validity – of the results.

Another limitation of this study was the collection of a single urine sample, which may have led to exposure misclassification when there is significant intra-individual variability. This type of exposure misclassification would be non-differential, and can bias the results toward or away from the null. Since the exposure variable was considered as a continuous entity in this analysis, the direction of the bias would depend on the population exposure level distribution (i.e. the distribution of subjects at varying exposure levels from

low to high exposure levels).As mentioned earlier, the collection of urine at multiple time points may provide a more accurate representation of exposure.

Another inherent limitation with this study is the accuracy of AGD measurement, as it likely influenced by measurement error (also likely non-differential). There can be issues in measuring such small distances, which can be even more difficult when they are being conducted in newborn infants. Moreover, this measurement can be somewhat more difficult in female infants. In males, the points of measurement (cephalad base of the penis, scrotum and anus) are relatively easy to identify, but in females it is not as clear (18). Lastly, examiners measuring the AGD in infants need to be made of aware of potential confounding due to perineal pathology, which includes the presence of edema, inflammation, cysts, fissures or fistulas, all of which can lead to measurement error (90).

Additionally, there may be the presence of inter-rater and intra-rater measurement errors, for the measurement of the AGD and the 2D:4D digit ratio, although steps were taken to try to minimize these errors such as taking multiple measurements, and providing standardized training and text material to all research staff. However, if these errors were present, they would be non-differential. Although not feasible in a large, nation-wide cohort study, measurement of the AGD and 2D:4D digit ratio by a single examiner would, at the very least, diminish the inter-rater measurement error. Further, it was reported that inter-rater variability in the measurement of the AGD is larger than the intra-rater variability (121,122).

Two potentially important variables that were not considered in this analysis were gestational age at time of maternal urine collection and the recruitment centre. In the TIDES study, the associations between AGD measures and many of the covariates included in the analysis differed by study centre; this were determined by conducting stratified analyses (76).

There is some evidence in the literature that suggests that there are adverse effects on infant birth weight following exposure to phthalates and BPA (see section 2.3); thus, infant weight may actually lie on the causal pathway between EDC exposure and the AGD outcome. This would mean birth weight is not a confounding but rather a mediating variable, and thus helps explain the relationship between the exposure and outcome variables. Thus, future analyses should potentially only include infant weight-for-length z score in order to control for infant birth weight and length. The most recent study by Swan et al. (2015) accounted for infant body size by using this z-score only(76).This study did not include it because it was taken out when conducting stepwise regression analysis.

Lastly, there may be a presence of residual confounding. This is a relatively new area of research and it is not known what influential variables may not have been collected and/or are missing from the analysis.

CHAPTER 6: CONCLUSIONS

This thesis was the first to examine the possible reproductive risks of exposure to priority environmental chemicals in a population of Canadian mothers and their infants. More specifically, it was the first study to examine the association between prenatal, maternal exposure to phthalates, BPA and TCS and the AGD and 2D:4D digit ratio in infants, in a Canadian setting.

The results of this study indicated that in female infants the phthalate metabolite MBzP was determined to be negatively associated with the anus-clitoris distance ($p=0.002$) and positively associated with the 2D:4D digit ratio ($p=0.024$). Furthermore, the phthalate metabolite MEP was positively associated with the anus-clitoris distance ($p=0.008$). In male infants, the phthalate metabolite MEHHP and total BPA were negatively associated with the 2D:4D digit ratio ($p=0.021$ and $p=0.002$, respectively). However, of the 12 exposure and 8 outcome variables considered (96 comparisons in total), only 5 were statistically significant. As 5 statistically significant results out of 96 tests in total are what exactly one would expect to see from Type I error, it is very unlikely that any of the abovementioned results represent a true effect. In addition, there is no consistency between the 5 associations seen and those that one would expect to see based on the proposed hypotheses (and current available evidence); and further, there was no correlation between the p-values of the various models. Moreover, as presented in the Chapter 2, there is a large inconsistency in published studies on the effects of phthalates on the AGD particularly in male infants, especially for the DEHP metabolites, in addition to the phthalate metabolites MBzP and MEP.

In conclusion, this study is one of the largest, well designed studies conducted to date that has investigated these specific environmental chemicals and respective outcomes. The

results did not provide strong evidence of an association between prenatal exposure to phthalates, BPA and TCS and the AGD and 2D:4D digit ratio, especially at the level that these chemicals have been detected in this Canadian pregnancy cohort. This information can be very informative to consumers, risk assessors, policy makers, and health care providers alike.

REFERENCES

1. Sweeney T. Is exposure to endocrine disrupting compounds during fetal/post-natal development affecting the reproductive potential of farm animals? Domest Anim Endocrinol [Internet]. 2002 Jul [cited 2013 Aug 22];23(1-2):203–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12142238
2. Kavlock RJ, Daston GP, DeRosa C, Fenner-Crisp P, Gray LE, Kaattari S, et al. Research needs for the risk assessment of health and environmental effects of endocrine disruptors: a report of the U.S. EPA-sponsored workshop. Environ Health Perspect [Internet]. 1996 Aug [cited 2013 Aug 22];104:715–40. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1469675&tool=pmcentrez&rendertype=abstract
3. Diamanti-Kandarakis E, Bourguignon J-P, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev [Internet]. 2009 Jun [cited 2013 Aug 18];30(4):293–342. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2726844&tool=pmcentrez&rendertype=abstract
4. Mendes AJ. J. The endocrine disrupters: a major medical challenge. Food Chem Toxicol [Internet]. 2002 Jun [cited 2013 Aug 22];40(6):781–8. Available from: http://dx.doi.org/10.1016/S0278-6915(02)00018-2
5. Bigsby R, Chapin RE, Daston GP, Davis BJ, Gorski J, Gray LE, et al. Evaluating the effects of endocrine disruptors on endocrine function during development. Environ Health Perspect [Internet]. 1999 Aug [cited 2013 Aug 22];107:613–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1567510&tool=pmcentrez&rendertype=abstract
6. Clayton EMR, Todd M, Dowd JB, Aiello AE. The impact of bisphenol A and triclosan on immune parameters in the U.S. population, NHANES 2003-2006. Environ Health Perspect [Internet]. 2011 Mar [cited 2013 Aug 17];119(3):390–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3060004&tool=pmcentrez&rendertype=abstract
7. Gore AC. Endocrine-disrupting chemicals: from basic research to clinical practice. [Internet]. Totowa, NJ. Totowa, New Jersey: Human Press; 2007 [cited 2013 Aug 21]. Available from: http://books.google.com/books?hl=en&lr=&id=-aQQAqW2YKYC&oi=fnd&pg=PR2&dq=Endocrine-disrupting+chemicals:+from+basic+research+to+clinical+practice&ots=6afvMmWEFl&sig=LUj5iNmZMY2XULRkFiZV8N2Rh0I
8. Arbuckle TE, Hauser R, Swan SH, Mao CS, Longnecker MP, Main KM, et al. Meeting report: measuring endocrine-sensitive endpoints within the first years of life. Environ Health Perspect [Internet]. 2008 Jul [cited 2013 Aug 21];116(7):948–51. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2453165&tool=pmcentrez&rendertype=abstract
9. Welsh M, Saunders PTK, Fisken M, Scott HM, Hutchison GR, Smith LB, et al. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. J Clin Invest [Internet]. 2008 Apr [cited 2015 Jan 22];118(4):1479–90. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2267017&tool=pmcentrez&rendertype=abstract
10. Wolf C, Lambright C, Mann P, Price M, Cooper RL, Ostby J, et al. Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, p,p′-DDE, and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169, and ethane dimethane sulphonate) during sexual differen. Toxicol Ind Health [Internet]. 1999 [cited 2013 Aug 22];15(1-2):94–118. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10188194
11. Longnecker MP, Gladen BC, Cupul-Uicab LA, Romano-Riquer P, Weber J-P, Chapin RE, et al. In utero exposure to the antiandrogen 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (DDE) in relation to anogenital distance in male newborns from Chiapas, México. Am J Epidemiol [Internet]. 2007 May 1 [cited 2013 Aug 21];165(9):1015–22. Available from:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1852527&tool=pmcentrez&rendertype=abstract
12. Salazar-Martinez E, Romano-Riquer P, Yanez-Marquez E, Longnecker MP, Hernández-Avila M. Anogenital distance in human male and female newborns: a descriptive, cross-sectional study. Environ Heal a Glob access Sci source [Internet]. 2004 Jan [cited 2013 Aug 22];3(8). Available from: http://www.ehjournal.net/content/3/1/8
13. Suzuki Y, Yoshinaga J, Mizumoto Y, Serizawaà S, Shiraishi H. Foetal exposure to phthalate esters and anogenital distance in male newborns. Int J Androl [Internet]. 2011 [cited 2013 Aug 11];35(3):236–44. Available from: http://resolver.scholarsportal.info/resolve/01056263/v35i0003/236_fetpeaadimn.xml
14. Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, et al. Decrease in Anogenital Distance among Male Infants with Prenatal Phthalate Exposure. Environ Health Perspect [Internet]. 2005 May 27 [cited 2013 Aug 21];113(8):1056–61. Available from: http://www.ehponline.org/ambra-doi-resolver/10.1289/ehp.8100
15. Swan SH. Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environ Res. 2008;108(2):177–84.
16. Callegari C, Everett S, Ross M, Brasel JA. Anogenital ratio: measure of fetal virilization in premature and full-term newborn infants. J Pediatr [Internet]. 1987 Aug [cited 2013 Aug 22];111(2):240–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/3612396
17. Phillip M, De Boer C, Pilpel D, Karplus M, Sofer S. Clitoral and penile sizes of full term newborns in two different ethnic groups. J Pediatr Endocrinol Metab [Internet]. 1996 [cited 2013 Aug 22];9(2):175–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8887140
18. Papadopoulou E, Vafeiadi M, Agramunt S, Basagaña X, Mathianaki K, Karakosta P, et al. Anogenital distances in newborns and children from Spain and Greece: predictors, tracking and reliability. Paediatr Perinat Epidemiol [Internet]. 2013 Jan [cited 2013 Aug 22];27(1):89–99. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23215716
19. Thankamony A, Ong KK, Dunger DB, Acerini CL, Hughes IA. Anogenital distance from birth to 2 years: a population study. Environ Health Perspect [Internet]. 2009 Nov [cited 2013 Aug 21];117(11):1786–90. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2801188&tool=pmcentrez&rendertype=abstract
20. Yeh S, Tsai M-Y, Xu Q, Mu X-M, Lardy H, Huang K-E, et al. Generation and characterization of androgen receptor knockout (ARKO) mice: an in vivo model for the study of androgen functions in selective tissues. Proc Natl Acad Sci U S A [Internet]. 2002 Oct 15 [cited 2013 Aug 22];99(21):13498–503. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=129702&tool=pmcentrez&rendertype=abstract
21. Mendiola J, Stahlhut RW, Jørgensen N, Liu F, Swan SH. Shorter anogenital distance predicts poorer semen quality in young men in Rochester, New York. Environ Health Perspect [Internet]. 2011 Jul [cited 2014 Jun 16];119(7):958–63. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3222997&tool=pmcentrez&rendertype=abstract
22. Eisenberg ML, Jensen TK, Walters RC, Skakkebaek NE, Lipshultz LI. The relationship between anogenital distance and reproductive hormone levels in adult men. J Urol [Internet]. 2012 Feb [cited 2015 Jan 22];187(2):594–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22177168
23. Eisenberg ML, Hsieh MH, Walters RC, Krasnow R, Lipshultz LI. The relationship between anogenital distance, fatherhood, and fertility in adult men. PLoS One [Internet]. 2011 Jan [cited 2015 Feb 12];6(5):e18973. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3092750&tool=pmcentrez&rendertype=abstract
24. Mendiola J, Roca M, Mínguez-Alarcón L, Mira-Escolano M-P, López-Espín JJ, Barrett ES, et al. Anogenital distance is related to ovarian follicular number in young Spanish women: a cross-sectional study. Environ Health [Internet]. 2012 Jan [cited 2015 Feb 20];11:90. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3562168&tool=pmcentrez&rendertype=abstract

25. Brown WM, Hines M, Fane BA, Breedlove SM. Masculinized finger length patterns in human males and females with congenital adrenal hyperplasia. Horm Behav [Internet]. 2002 Dec [cited 2013 Aug 22];42(4):380–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12488105
26. Buck JJ, Williams RM, Hughes IA, Acerini CL. In-utero androgen exposure and 2nd to 4th digit length ratio-comparisons between healthy controls and females with classical congenital adrenal hyperplasia. Hum Reprod [Internet]. 2003 May [cited 2013 Aug 22];18(5):976–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12721171
27. Hönekopp J, Manning JT, Müller C. Digit ratio (2D:4D) and physical fitness in males and females: Evidence for effects of prenatal androgens on sexually selected traits. Horm Behav [Internet]. 2006 Apr [cited 2013 Aug 22];49(4):545–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16403410
28. McIntyre MH. The use of digit ratios as markers for perinatal androgen action. Reprod Biol Endocrinol [Internet]. 2006 Jan [cited 2013 Aug 7];4(10). Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1409789&tool=pmcentrez&rendertype=abstract
29. Okten A, Kalyoncu M, Yariş N. The ratio of second- and fourth-digit lengths and congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Early Hum Dev [Internet]. 2002 Dec [cited 2013 Aug 22];70(1-2):47–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12441204
30. Manning JT, Scutt D, Wilson J, Lewis-Jones DI. The ratio of 2nd to 4th digit length: a predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. Hum Reprod Embryol [Internet]. 1998 Nov [cited 2013 Aug 22];13(11):3000–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9853845
31. Manning JT, Barley L, Walton J, Lewis-Jones DI, Trivers RL, Singh D, et al. The 2nd:4th digit ratio, sexual dimorphism, population differences, and reproductive success: evidence for sexually antagonistic genes? Evol Hum Behav [Internet]. 2000 May 1 [cited 2013 Aug 22];21(3):163–83. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10828555
32. Zheng Z, Cohn MJ. Developmental basis of sexually dimorphic digit ratios. Proc Natl Acad Sci U S A [Internet]. 2011 Sep 27 [cited 2013 Aug 21];108(39):16289–94. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3182741&tool=pmcentrez&rendertype=abstract
33. Lutchmaya S, Baron-Cohen S, Raggatt P, Knickmeyer R, Manning JT. 2nd to 4th digit ratios, fetal testosterone and estradiol. Early Hum Dev [Internet]. 2004 Apr [cited 2013 Aug 13];77(1-2):23–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15113628
34. Klimek M, Galbarczyk A, Nenko I, Alvarado LC, Jasienska G. Digit ratio (2D:4D) as an indicator of body size, testosterone concentration and number of children in human males. Ann Hum Biol [Internet]. 2014 [cited 2015 Mar 29];41(6):518–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24766144
35. Thompson RC, Moore CJ, vom Saal FS, Swan SH. Plastics, the environment and human health: current consensus and future trends. Philos Trans R Soc London Ser B, Biol Ph [Internet]. 2009 Jul 27 [cited 2013 Aug 7];364(1526):2153–66. Available from: http://rstb.royalsocietypublishing.org/content/364/1526/2153.short
36. Kay VR, Chambers C, Foster WG. Reproductive and developmental effects of phthalate diesters in females. Crit Rev Toxicol [Internet]. 2013 Mar [cited 2015 Jan 30];43(3):200–19. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3604737&tool=pmcentrez&rendertype=abstract
37. Howdeshell KL, Rider C V, Wilson VS, Gray LE. Mechanisms of action of phthalate esters, individually and in combination, to induce abnormal reproductive development in male laboratory rats. Environ Res [Internet]. 2008 Oct [cited 2015 Jan 30];108(2):168–76. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18949836
38. Lyche JL, Gutleb AC, Bergman A, Eriksen GS, Murk AJ, Ropstad E, et al. Reproductive and developmental toxicity of phthalates. J Toxicol Environ Health B Crit Rev [Internet]. 2009 Apr [cited 2014 Jul 28];12(4):225–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20183522
39. Braun JM, Sathyanarayana S, Hauser R. Phthalate exposure and children's health. Curr Opin Pediatr [Internet]. 2013 Apr [cited 2013 Nov 14];25(2):247–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23429708
40. Marsee K, Woodruff TJ, Axelrad DA, Calafat AM, Swan SH. Estimated daily phthalate exposures in a population of mothers of male infants exhibiting reduced anogenital distance. Environ Health Perspect

[Internet]. 2006 Jun [cited 2014 Jun 16];114(6):805–9. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1480516&tool=pmcentrez&rendertype=abstract
41. Swan SH. Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environ Res [Internet]. 2008 Oct [cited 2014 Jun 16];108(2):177–84. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2775531&tool=pmcentrez&rendertype=abstract
42. Jeng HA. Exposure to endocrine disrupting chemicals and male reproductive health. Front public Heal [Internet]. 2014 Jan [cited 2014 Jul 10];2:55. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4046332&tool=pmcentrez&rendertype=abstract
43. Health Canada, Healthy Environments and Consumer Safety Branch, Environmental and Radiation Health Sciences Directorate, Chemicals Surveillance Bureau NBD. Second Report on Human Biomonitoring of Environmental Chemicals in Canada [Health Canada, 2013] [Internet]. 2013 Apr. Available from: http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/chms-ecms-cycle2/index-eng.php
44. Welsh M, Saunders PTK, Fisken M, Scott HM, Hutchison GR, Smith LB, et al. Identification in rats of a programming window for reproductive tract masculinization, disruption of which leads to hypospadias and cryptorchidism. J Clin Invest [Internet]. American Society for Clinical Investigation; 2008 Apr 1 [cited 2013 Aug 22];118(4):1479–90. Available from: http://www.jci.org/articles/view/34241
45. Akingbemi BT, Ge R, Klinefelter GR, Zirkin BR, Hardy MP. Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances. Proc Natl Acad Sci U S A [Internet]. 2004 Jan 20 [cited 2013 Aug 22];101(3):775–80. Available from: http://www.pnas.org/content/101/3/775.long
46. Borch J, Dalgaard M, Ladefoged O. Early testicular effects in rats perinatally exposed to DEHP in combination with DEHA--apoptosis assessment and immunohistochemical studies. Reprod Toxicol [Internet]. 2004 [cited 2013 Aug 22];19(4):517–25. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15749266
47. Foster P. Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters. Int J Androl [Internet]. 2006 Feb [cited 2013 Aug 22];29(1):140–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16102138
48. Gray LE, Wilson VS, Stoker TE, Lambright C, Furr J, Noriega N, et al. Adverse effects of environmental antiandrogens and androgens on reproductive development in mammals. Int J Androl. 2006 Feb;29(1):96–104.
49. Health Canada, Healthy Environments and Consumer Safety Branch, Food Directorate, Chemicals Safety Bureau FRD. Bisphenol A - Packaging Materials - Food Safety - Health Canada [Internet]. 2004 [cited 2015 Mar 22]. Available from: http://www.hc-sc.gc.ca/fn-an/securit/packag-emball/bpa/index-eng.php
50. Meeker JD, Sathyanarayana S, Swan SH. Phthalates and other additives in plastics: human exposure and associated health outcomes. Philos Trans R Soc Lond B Biol Sci [Internet]. 2009 Jul 27 [cited 2013 Nov 14];364(1526):2097–113. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2873014&tool=pmcentrez&rendertype=abstract
51. Peretz J, Vrooman L, Ricke WA, Hunt PA, Ehrlich S, Hauser R, et al. Bisphenol A and Reproductive Health: Update of Experimental and Human Evidence, 2007-2013. Environ Health Perspect [Internet]. 2014 [cited 2014 Jun 13]; Available from: http://ehp.niehs.nih.gov/wp-content/uploads/advpub/2014/6/ehp.1307728.pdf
52. Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons W V. Human exposure to bisphenol A (BPA). Reprod Toxicol [Internet]. 2007 [cited 2013 Aug 7];24(2):139–77. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17825522
53. Geens T, Goeyens L, Kannan K, Neels H, Covaci A. Levels of bisphenol-A in thermal paper receipts from Belgium and estimation of human exposure. Sci Total Environ [Internet]. 2012 Oct 1 [cited 2015 Mar 22];435-436:30–3. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22846760
54. Brotons JA, Olea-Serrano MF, Villalobos M, Pedraza V, Olea N. Xenoestrogens released from lacquer coatings in food cans. Environ Health Perspect [Internet]. 1995 Jun [cited 2015 Feb 4];103(6):608–12. Available from:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1519121&tool=pmcentrez&rendertype=abstract
55. Matsumoto H, Adachi S, Suzuki Y. Bisphenol A in ambient air particulates responsible for the proliferation of MCF-7 human breast cancer cells and Its concentration changes over 6 months. Arch Environ Contam Toxicol [Internet]. 2005 May [cited 2015 Feb 4];48(4):459–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15883673
56. Rodriguez-Mozaz S, de Alda ML, Barceló D. Analysis of bisphenol A in natural waters by means of an optical immunosensor. Water Res [Internet]. 2005 Dec [cited 2015 Feb 4];39(20):5071–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16337256
57. Bushnik T, Haines D, Levallois P, Levesque J, Van Oostdam J, Viau C. Lead and bisphenol A concentrations in the Canadian population. Heal reports [Internet]. 2010 Sep [cited 2015 Jan 26];21(3):7–18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20973429
58. Cantonwine D, Meeker JD, Hu H, Sánchez BN, Lamadrid-Figueroa H, Mercado-García A, et al. Bisphenol a exposure in Mexico City and risk of prematurity: a pilot nested case control study. Environ Health [Internet]. 2010 Jan [cited 2015 Mar 29];9(1):62. Available from: http://www.ehjournal.net/content/9/1/62
59. Weinberger B, Vetrano AM, Archer FE, Marcella SW, Buckley B, Wartenberg D, et al. Effects of maternal exposure to phthalates and bisphenol A during pregnancy on gestational age. J Matern Fetal Neonatal Med [Internet]. Informa UK Ltd. London; 2014 Mar 20 [cited 2015 Mar 29];27(4):323–7. Available from: http://informahealthcare.com/doi/abs/10.3109/14767058.2013.815718
60. Chou W-C, Chen J-L, Lin C-F, Chen Y-C, Shih F-C, Chuang C-Y. Biomonitoring of bisphenol A concentrations in maternal and umbilical cord blood in regard to birth outcomes and adipokine expression: a birth cohort study in Taiwan. Environ Health [Internet]. 2011 Jan [cited 2015 Mar 29];10:94. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3225308&tool=pmcentrez&rendertype=abstract
61. Miao M, Yuan W, Zhu G, He X, Li D-K. In utero exposure to bisphenol-A and its effect on birth weight of offspring. Reprod Toxicol [Internet]. 2011 Jul [cited 2015 Feb 4];32(1):64–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21440056
62. Padmanabhan V, Siefert K, Ransom S, Johnson T, Pinkerton J, Anderson L, et al. Maternal bisphenol-A levels at delivery: a looming problem? J Perinatol [Internet]. 2008 Apr [cited 2015 Mar 29];28(4):258–63. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4033524&tool=pmcentrez&rendertype=abstract
63. Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, et al. Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect [Internet]. 2008 Aug [cited 2013 Aug 10];116(8):1092–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2516577&tool=pmcentrez&rendertype=abstract
64. Cabaton NJ, Wadia PR, Rubin BS, Zalko D, Schaeberle CM, Askenase MH, et al. Perinatal exposure to environmentally relevant levels of bisphenol A decreases fertility and fecundity in CD-1 mice. Environ Health Perspect [Internet]. 2011 Apr [cited 2015 Feb 22];119(4):547–52. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3080939&tool=pmcentrez&rendertype=abstract
65. Allmyr M, Panagiotidis G, Sparve E, Diczfalusy U, Sandborgh-Englund G. Human exposure to triclosan via toothpaste does not change CYP3A4 activity or plasma concentrations of thyroid hormones. Basic Clin Pharmacol Toxicol [Internet]. 2009 Nov [cited 2013 Aug 22];105(5):339–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19686543
66. Kisok K, Park H, Yang W, Lee JH. Urinary concentrations of bisphenol A and triclosan and associations with demographic factors in the Korean population. Environ Res [Internet]. 2011 Nov [cited 2013 Aug 22];111(8):1280–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21925656
67. Dann AB, Hontela A. Triclosan: environmental exposure, toxicity and mechanisms of action. J Appl Toxicol [Internet]. 2011 May [cited 2015 Mar 10];31(4):285–311. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21462230

68. Vélez MP, Arbuckle TE, Fraser WD. Female exposure to phenols and phthalates and time to pregnancy: the Maternal-Infant Research on Environmental Chemicals (MIREC) Study. Fertil Steril [Internet]. 2015 Feb 11 [cited 2015 Mar 22]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/25681860
69. Philippat C, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, et al. Exposure to phthalates and phenols during pregnancy and offspring size at birth. Environ Health Perspect [Internet]. 2012 Mar [cited 2013 Aug 22];120(3):464–70. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3295340&tool=pmcentrez&rendertype=abstract
70. Lankester J, Patel C, Cullen MR, Ley C, Parsonnet J. Urinary triclosan is associated with elevated body mass index in NHANES. PLoS One [Internet]. 2013 Jan [cited 2015 Mar 22];8(11):e80057. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3836985&tool=pmcentrez&rendertype=abstract
71. Zorrilla LM, Gibson EK, Jeffay SC, Crofton KM, Setzer WR, Cooper RL, et al. The effects of triclosan on puberty and thyroid hormones in male Wistar rats. Toxicol Sci [Internet]. 2009 Jan [cited 2013 Aug 21];107(1):56–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18940961
72. Gee RH, Charles A, Taylor N, Darbre PD. Oestrogenic and androgenic activity of triclosan in breast cancer cells. J Appl Toxicol [Internet]. 2008 [cited 2013 Aug 21];28:78–91. Available from: http://onlinelibrary.wiley.com/doi/10.1002/jat.1316/abstract
73. Bornehag C-G, Carlstedt F, Jönsson BA, Lindh CH, Jensen TK, Bodin A, et al. Prenatal phthalate exposures and anogenital distance in Swedish boys. Environ Health Perspect [Internet]. 2015 Jan [cited 2015 Feb 1];123(1):101–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4286276&tool=pmcentrez&rendertype=abstract
74. Bustamante-Montes LP, Hernández-Valero MA, Flores-Pimentel D, García-Fábila M, Amaya-Chávez A, Barr DB, et al. Prenatal exposure to phthalates is associated with decreased anogenital distance and penile size in male newborns. J Dev Orig Health Dis [Internet]. 2013 Aug [cited 2014 Jun 16];4(4). Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3862078&tool=pmcentrez&rendertype=abstract
75. Huang P-C, Kuo P-L, Chou Y-Y, Lin S-J, Lee C-C. Association between prenatal exposure to phthalates and the health of newborns. Environ Int [Internet]. 2009 Jan [cited 2014 Jun 2];35(1):14–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18640725
76. Swan SH, Sathyanarayana S, Barrett ES, Janssen S, Liu F, Nguyen RHN, et al. First trimester phthalate exposure and anogenital distance in newborns. Hum Reprod [Internet]. 2015 Feb 18 [cited 2015 Mar 5];(hu). Available from: http://www.ncbi.nlm.nih.gov/pubmed/25697839
77. Miao M, Yuan W, He Y, Zhou Z, Wang J, Gao E, et al. In utero exposure to bisphenol-A and anogenital distance of male offspring. Birth Defects Res A Clin Mol Teratol [Internet]. 2011 Oct [cited 2013 Aug 21];91(10):867–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21987463
78. Health Canada, Healthy Environments and Consumer Safety Branch, Environmental and Radiation Health Sciences Directorate, Chemicals Surveillance Bureau NBD. Human Biomonitoring of Environmental Chemicals - Health Canada [Internet]. 2010 [cited 2015 Mar 23]. Available from: http://www.hc-sc.gc.ca/ewh-semt/contaminants/human-humaine/index-eng.php
79. Koch HM, Angerer J. Phthalates: Biomarkers and Human Biomonitoring. 2012.
80. Haines DA, Arbuckle TE, Lye E, Legrand M, Fisher M, Langlois R, et al. Reporting results of human biomonitoring of environmental chemicals to study participants: a comparison of approaches followed in two Canadian studies. J Epidemiol Community Health [Internet]. 2011 Mar [cited 2014 Jun 16];65(3):191–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20628082
81. Shea KM. Biomarkers and Human Biomonitoring [Internet]. World Health Organization. 2011 [cited 2015 Feb 9]. Available from: http://www.who.int/ceh/capacity/biomarkers.pdf
82. Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect [Internet]. 2005 Feb [cited 2015 Mar 23];113(2):192–200. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1277864&tool=pmcentrez&rendertype=abstract

83. Adibi JJ, Whyatt RM, Williams PL, Calafat AM, Camann D, Herrick R, et al. Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. Environ Health Perspect [Internet]. 2008 Apr [cited 2015 Mar 23];116(4):467–73. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2291011&tool=pmcentrez&rendertype=abstract
84. Fisher M, Arbuckle TE, Mallick R, LeBlanc A, Hauser R, Feeley M, et al. Bisphenol A and phthalate metabolite urinary concentrations: Daily and across pregnancy variability. J Expo Sci Environ Epidemiol [Internet]. 2014 Sep 24 [cited 2015 Jan 30]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/25248937
85. Arbuckle TE. Maternal-infant biomonitoring of environmental chemicals: the epidemiologic challenges. Birth Defects Res A Clin Mol Teratol [Internet]. 2010 Oct [cited 2015 Jan 30];88(10):931–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20706992
86. Government of Canada HC. Overview of the Chemicals Management Plan [Internet]. 2009 [cited 2015 Feb 22]. Available from: http://www.chemicalsubstanceschimiques.gc.ca/fact-fait/overview-vue-eng.php
87. Provencher G, Bérubé R, Dumas P, Bienvenu J-F, Gaudreau E, Bélanger P, et al. Determination of bisphenol A, triclosan and their metabolites in human urine using isotope-dilution liquid chromatography-tandem mass spectrometry. J Chromatogr A [Internet]. 2014 Jun 27 [cited 2015 Feb 22];1348:97–104. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24835763
88. Arbuckle TE, Davis K, Marro L, Fisher M, Legrand M, LeBlanc A, et al. Phthalate and bisphenol A exposure among pregnant women in Canada - Results from the MIREC study. Environ Int [Internet]. 2014 Jul [cited 2014 May 26];68:55–65. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24709781
89. Sathyanarayana S, Beard L, Zhou C, Grady R. Measurement and correlates of ano-genital distance in healthy, newborn infants. Int J Androl [Internet]. 2010 Apr [cited 2015 Feb 22];33(2):317–23. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2943951&tool=pmcentrez&rendertype=abstract
90. Dean A, Sharpe RM. Anogenital distance or digit length ratio as measures of fetal androgen exposure: relationship to male reproductive development and its disorders. J Clin Endocrinol Metab [Internet]. 2013 Jun 8 [cited 2013 Aug 22];98(6):2230–8. Available from: http://jcem.endojournals.org/content/early/2013/04/05/jc.2012-4057
91. Fowler PA, Bhattacharya S, Flannigan S, Drake AJ, O'Shaughnessy PJ. Maternal cigarette smoking and effects on androgen action in male offspring: unexpected effects on second-trimester anogenital distance. J Clin Endocrinol Metab [Internet]. 2011 Sep [cited 2013 Aug 21];96(9):E1502–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21752894
92. Gyekis J, Anthony K, Foreman JE, Klein LC, Vandenbergh DJ. Perinatal nicotine exposure delays genital development in mice. Reprod Toxicol [Internet]. 2010 Jun [cited 2013 Aug 27];29(3):378–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20080174
93. Rizwan S, Manning JT, Brabin BJ. Maternal smoking during pregnancy and possible effects of in utero testosterone: evidence from the 2D:4D finger length ratio. Early Hum Dev [Internet]. 2007 Feb [cited 2013 Aug 27];83(2):87–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16814493
94. World Health Organization, Child Growth Standards [Internet]. WHO Anthro (version 3.2.2, January 2011) and macros. World Health Organization; 2011 [cited 2015 Jan 31]. Available from: http://www.who.int/childgrowth/software/en/
95. Helsel DR. Statistics for Censored Environmental Data Using Minitab® and R [Internet]. Hoboken, New Jersey: John Wiley and Sons; 2012 [cited 2013 Aug 28]. Available from: http://onlinelibrary.wiley.com.proxy.bib.uottawa.ca/book/10.1002/9781118162729?systemMessage=Wiley+Online+Library+will+be+disrupted+on+31+August+from+10:00-12:00+BST+(05:00-07:00+EDT)+for+essential+maintenance
96. Miller RC, Brindle E, Holman DJ, Shofer J, Klein NA, Soules MR, et al. Comparison of specific gravity and creatinine for normalizing urinary reproductive hormone concentrations. Clin Chem [Internet]. 2004 May [cited 2013 Aug 22];50(5):924–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15105350
97. Haddow JE, Knight GJ, Palomaki GE, Neveux LM, Chilmonczyk BA. Replacing creatinine measurements with specific gravity values to adjust urine cotinine concentrations. Clin Chem [Internet].

1994 Apr [cited 2015 Mar 23];40(4):562–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8149610
98. Boeniger MF, Lowry LK, Rosenberg J. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. Am Ind Hyg Assoc J [Internet]. 1993 Oct [cited 2015 Mar 23];54(10):615–27. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8237794
99. Hauser R, Calafat AM. Phthalates and human health. Occup Environ Med [Internet]. 2005 Nov [cited 2015 Mar 23];62(11):806–18. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1740925&tool=pmcentrez&rendertype=abstract
100. Sathyanarayana S. Phthalates and children's health. Curr Probl Pediatr Adolesc Health Care [Internet]. 2008 Feb [cited 2015 Mar 23];38(2):34–49. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18237855
101. Schettler T. Human exposure to phthalates via consumer products. Int J Androl [Internet]. 2006 Feb [cited 2015 Mar 23];29(1):134–9; discussion 181–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16466533
102. Barrett ES, Parlett LE, Redmon JB, Swan SH. Evidence for sexually dimorphic associations between maternal characteristics and anogenital distance, a marker of reproductive development. Am J Epidemiol [Internet]. 2014 Jan 1 [cited 2014 Jun 16];179(1):57–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24124194
103. Arbuckle TE, Marro L, Davis K, Fisher M, Ayotte P, Bélanger P, et al. Exposure to Free and Conjugated Forms of Bisphenol A and Triclosan among Pregnant Women in the MIREC Cohort. Environ Health Perspect [Internet]. 2014 Nov 21 [cited 2015 Jan 30]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/25494523
104. Brock JW, Yoshimura Y, Barr JR, Maggio VL, Graiser SR, Nakazawa H, et al. Measurement of bisphenol A levels in human urine. J Expo Anal Environ Epidemiol [Internet]. 2001 [cited 2015 Feb 1];11(4):323–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11571611
105. Fukata H, Miyagawa H, Yamazaki N, Mori C. Comparison of Elisa- and LC-MS-Based Methodologies for the Exposure Assessment of Bisphenol A. Toxicol Mech Methods [Internet]. 2006 Jan [cited 2015 Feb 1];16(8):427–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20021017
106. Kim Y-H, Kim C-S, Park S, Han SY, Pyo M-Y, Yang M. Gender differences in the levels of bisphenol A metabolites in urine. Biochem Biophys Res Commun [Internet]. 2003 Dec 12 [cited 2015 Feb 1];312(2):441–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14637157
107. Völkel W, Colnot T, Csanády GA, Filser JG, Dekant W. Metabolism and kinetics of bisphenol a in humans at low doses following oral administration. Chem Res Toxicol [Internet]. 2002 Oct [cited 2015 Jan 14];15(10):1281–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12387626
108. Engel SM, Zhu C, Berkowitz GS, Calafat AM, Silva MJ, Miodovnik A, et al. Prenatal phthalate exposure and performance on the Neonatal Behavioral Assessment Scale in a multiethnic birth cohort. Neurotoxicology [Internet]. 2009 Jul [cited 2015 Feb 1];30(4):522–8. Available from: http://www.sciencedirect.com/science/article/pii/S0161813X09000837
109. Casas L, Fernández MF, Llop S, Guxens M, Ballester F, Olea N, et al. Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children. Environ Int [Internet]. 2011 Jul [cited 2015 Feb 1];37(5):858–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21440302
110. Lakind JS, Levesque J, Dumas P, Bryan S, Clarke J, Naiman DQ. Comparing United States and Canadian population exposures from National Biomonitoring Surveys: bisphenol A intake as a case study. J Expo Sci Environ Epidemiol [Internet]. 2012 [cited 2015 Feb 1];22(3):219–26. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3331622&tool=pmcentrez&rendertype=abstract
111. Hallmark N, Walker M, McKinnell C, Mahood IK, Scott H, Bayne R, et al. Effects of monobutyl and di(n-butyl) phthalate in vitro on steroidogenesis and Leydig cell aggregation in fetal testis explants from the rat: comparison with effects in vivo in the fetal rat and neonatal marmoset and in vitro in the human. Environ Health Perspect [Internet]. 2007 Mar [cited 2015 Feb 8];115(3):390–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1849934&tool=pmcentrez&rendertype=abstract
112. Lambrot R, Muczynski V, Lécureuil C, Angenard G, Coffigny H, Pairault C, et al. Phthalates impair germ cell development in the human fetal testis in vitro without change in testosterone production.

Environ Health Perspect [Internet]. 2009 Jan [cited 2015 Feb 8];117(1):32–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2627862&tool=pmcentrez&rendertype=abstract
113. Matthews JB, Twomey K, Zacharewski TR. In vitro and in vivo interactions of bisphenol A and its metabolite, bisphenol A glucuronide, with estrogen receptors alpha and beta. Chem Res Toxicol [Internet]. 2001 Feb [cited 2015 Feb 4];14(2):149–57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11258963
114. Teeguarden JG, Calafat AM, Ye X, Doerge DR, Churchwell MI, Gunawan R, et al. Twenty-four hour human urine and serum profiles of bisphenol a during high-dietary exposure. Toxicol Sci [Internet]. 2011 Sep [cited 2015 Jan 19];123(1):48–57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21705716
115. Völkel W, Kiranoglu M, Fromme H. Determination of free and total bisphenol A in human urine to assess daily uptake as a basis for a valid risk assessment. Toxicol Lett [Internet]. 2008 Jul 10 [cited 2015 Feb 4];179(3):155–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18579321
116. Vandenberg LN, Chahoud I, Padmanabhan V, Paumgartten FJR, Schoenfelder G. Biomonitoring studies should be used by regulatory agencies to assess human exposure levels and safety of bisphenol A. Environ Health Perspect [Internet]. 2010 Aug [cited 2015 Feb 4];118(8):1051–4. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2920081&tool=pmcentrez&rendertype=abstract
117. Witorsch RJ. Critical analysis of endocrine disruptive activity of triclosan and its relevance to human exposure through the use of personal care products. Crit Rev Toxicol [Internet]. 2014 Jul [cited 2015 Feb 8];44(6):535–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24897554
118. Abbott AD, Colman RJ, Tiefenthaler R, Dumesic DA, Abbott DH. Early-to-mid gestation fetal testosterone increases right hand 2D:4D finger length ratio in polycystic ovary syndrome-like monkeys. PLoS One [Internet]. 2012 Jan [cited 2015 Feb 8];7(8):e42372. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3425513&tool=pmcentrez&rendertype=abstract
119. Manno FAM. Measurement of the digit lengths and the anogenital distance in mice. Physiol Behav [Internet]. 2008 Jan 28 [cited 2015 Feb 8];93(1-2):364–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18054055
120. Hurd PL, Bailey AA, Gongal PA, Yan RH, Greer JJ, Pagliardini S. Intrauterine position effects on anogenital distance and digit ratio in male and female mice. Arch Sex Behav [Internet]. 2008 Feb [cited 2015 Feb 8];37(1):9–18. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18080736
121. Romano-Riquer SP, Hernández-Avila M, Gladen BC, Cupul-Uicab LA, Longnecker MP. Reliability and determinants of anogenital distance and penis dimensions in male newborns from Chiapas, Mexico. Paediatr Perinat Epidemiol [Internet]. 2007 May [cited 2014 Jun 16];21(3):219–28. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3653615&tool=pmcentrez&rendertype=abstract
122. Liu C, Xu X, Huo X. Anogenital distance and its application in environmental health research. Environ Sci Pollut Res Int [Internet]. 2014 Apr [cited 2014 Jun 16];21(8):5457–64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24474565

APPENDICES

Appendix 1: Informed consent for the MIREC Study

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Pour l'amour des enfants

Université de Montréal

INFORMED CONSENT

MIREC
Maternal-Infant Research on Environmental Chemicals

Maternal-Infant Research on Environmental Chemicals (The MIREC Study): A National Profile of *In Utero* and Lactational Exposure to Environmental Contaminants

Investigators:

Co- principal investigators:

Dr William D. Fraser	Obstetrician, CHU Ste-Justine
Tye Arbuckle	Epidemiologist, Health Canada

Co-investigators:

Jean-Philippe Weber	Professor (retired), Université de Montréal
Melissa Legrand	Nutritionist, Health Canada
Premkumari Kumarathasan	Toxicologist, Health Canada
Renaud Vincent	Toxicologist, Health Canada
Kevin Cockell	Nutritionist, Health Canada
Maya Villeneuve	Nutritionist, Health Canada
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Zhong-Cheng Luo	Epidemiologist, CHU Ste-Justine
Adrienne Ettinger	Epidemiologist, Harvard
Robert Platt	Biostatistician, McGill University
Grant Mitchell	Geneticist, CHU Ste-Justine
Pierre Julien	Professor, Université Laval
Denise Avard	Professor, Université de Montréal
Nick Hidiroglou	Nutritionist, Health Canada
Hope Weiler	Nutritionist, McGill University
Alain Leblanc	Chemist, CTQ/INSPQ
Mandy Fisher	Epidemiologist, Health Canada
Monique D'Amour	Scientific advisor, Health Canada

Co-investigators from milk component (of Health Canada): Bob Dabeka, Thea Rawn, Xu-Liang Cao, Adam Becalski, Nimal Ratnayake, Genevieve Bondy, Dawn Jin, Zhongwen Wang, Eric Braekevelt.

Local Site Investigators: Peter von Dadelszen (Vancouver), Denise Hemmings and Jingwei Wang (Edmonton), Michael Helewa and Shayne Taback (Winnipeg), Mathew Sermer (Toronto), Warren G. Foster (Hamilton), Greg Ross and Paul Fredette (Sudbury), Graeme Smith (Kingston), Mark Walker (Ottawa), Roberta Shear (Montreal), William D. Fraser (Montreal) and Linda Dodds (Halifax).

Local Site Investigator: Dr. William D. Fraser, M.D., M.Sc., FRCSC

Funding agencies and research partners:
Health Canada
Ontario Ministry of the Environment
Canadian Institutes of Health Research

We are asking for your participation in a study on the effects of exposure to metals such as lead and mercury on pregnancy outcomes. The study will also measure the levels of various environmental chemicals in your blood, urine, hair and in your infant's cord blood and feces. We will also conduct a survey of nutrients, immune characteristics and environmental chemicals in breast milk. The information on breast milk together with the information during pregnancy could help improve the health of mothers and babies. We invite you to read this consent form to learn more about the study. Please feel free to ask the study personnel (local site investigator or research nurse) any questions which you may have.

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1. Why is the MIREC study being done?

Some recent reports have raised concerns about the number of chemicals in our bodies and what, if any, health effects may be associated with the levels measured. For some chemicals such as lead, government policies banning lead in gasoline and paint have helped to reduce blood lead levels in children. Lead may still persist in soil, dust and water. Work continues on assessing the health risks of low lead exposure in children.

Smoking in pregnancy has long been linked with a higher risk of low birth weight, and other harmful effects on the baby. Currently, there is little information about exposure to tobacco smoke among pregnant women in Canada. In particular, there is a lack of information on whether smoking behavior changes during pregnancy. Governments and public health workers could use this information to develop policies and programs that help pregnant women quit smoking and avoid exposure to second-hand tobacco smoke.

Most often breast feeding is the best method of feeding your infant. It gives good nutrition, immune protection and emotional benefits to your infant. There are also health benefits to

you when you breastfeed. This study will collect information on nutrients and environmental chemicals found in breast milk as well as, the immune protection that it provides to the baby. This study will be the largest ever conducted in Canada and will provide valuable information. Many other studies conducted on pregnant women in Canada have been very small or limited to certain locations. Information from this research may also assist in the development of nutrition programs and policy for breast-feeding women.

2. What is the purpose of this project?

There are three main purposes for this research study:

(1) to measure the extent to which pregnant women and their babies are exposed to environmental chemicals including; lead, mercury, cadmium, arsenic and manganese (heavy metals); persistent organic pollutants (POPs); pesticides; flame retardants; plastic softeners; surface coatings; food packaging and processing related chemicals and smoking by-products. Exposure will be measured in mother's blood, urine, hair, breast milk as well as cord blood and meconium (baby's first stool).

(2) to measure some of the beneficial components in human breast milk (nutrients and immune factors); and

(3) to assess what health risks, if any, are associated with the levels of heavy metals measured.

We know that not everyone who experiences the same risks will develop a certain health problem. Therefore, we will also test characteristics of women (genomic testing: to identify genes that could affect reactions to certain chemicals or diseases; nutritional status; and other markers), which might make mothers or their babies more or less likely to experience harmful effects from exposure to environmental chemicals.

3. Number of participants in this study

We aim to recruit 2,000 women in their first trimester of pregnancy from 8 to 10 sites across Canada.

4. What will be the process of this study?

If you agree to participate we will arrange our meetings with you during your regularly scheduled prenatal visits (6 – 13 weeks, 16 – 21 weeks, and 32 – 34 weeks) and at delivery, as well as a home visit 3 to 8 weeks after the birth of your child. During these visits you will be asked to:

a) Complete questionnaires.

The first questionnaire takes close to 45 minutes to complete. It will collect information on you and the baby's father including general health, any previous pregnancies, occupation, education, lifestyle, and potential sources of exposure to chemicals. At each visit during your pregnancy and at delivery we will ask you to complete some short follow-up questionnaires (about 10-20 *minutes*). Some of the questions will help us determine your nutritional status based on the foods you eat and your use of dietary supplements. The study nurse will

administer these questionnaires and help you to understand the questions if needed. You may refuse to answer any questions that make you uncomfortable.

b) Allow us access to your medical records to record information on the health of your pregnancy and your baby.
The information to be collected from your medical records includes any health problems you experienced during pregnancy (e.g., elevated blood pressure) and the health of your baby (e.g., birth weight).

c) Provide us with samples of your blood and urine.
At each visit (once in each trimester and at delivery) you will be asked to provide about 120 *ml* of urine (about ½ cup) and an extra 38 ml of blood (2.5 tablespoons).

d) Allow us to collect cord blood and meconium (baby's first stools).
The study will need to collect 100 *ml* (approximately 7 tablespoons) of cord blood and 20 grams (0.8 oz.) of meconium. Meconium is the first stools that your baby excretes over the 2 days following birth. The collections of these samples are non-invasive and safe to you and your baby.

e) When your baby is between 3 and 8 weeks of age a research nurse will call you to arrange a home visit. At this home visit you will be asked to:

- complete a 20 minute questionnaire about your diet, lifestyle and baby's feeding
- provide about 200 ml (about ¾ of cup) of your breast milk expressed by hand or with a breast pump. We will provide you with a kit for collecting the milk and step by step instructions. We will also give you contact information for the nurse and the site investigator if you have any questions.
- provide a small hair sample. The hair strands will be collected from the lower back of your head where it would not be noticeable. The nurses are trained to perform this task. (NOTE: If you do not wish to participate in the breast milk collection, we will request a hair sample when we collect your baby's meconium.)

5. What advantages and benefits can I expect?

In regards to communication of individual results, if you have a result that is higher than health- based guidelines, and there are preventive measures or treatments, you will be informed through your doctor. These individual results will be added to your medical chart.

We will also provide you with some material to help you understand these chemicals. You should also know that it could be several months to years after your pregnancy before all the chemical results will be available, given the time and costs for these analyses.

For the first time in Canada we will have some measures of levels of important environmental chemicals in pregnant women and their babies that can be used as a baseline and to direct further research. This research may also help to develop useful recommendations for pregnant women to reduce the risk of poor pregnancy outcomes associated with pollutants in their environment and identify potential sources of exposure.

This study will produce new knowledge on smoking behaviour, use of methods to quit smoking, which are being encouraged for use during pregnancy, and smoking restrictions in the home.

6. What are the possible risks for me or my baby?

There are minimal risks to you or your baby. There are no additional medications or treatments for this study. We will ask you to donate an extra 38 ml (2.5 tablespoons) of blood at first visit, and at each subsequent prenatal visit and at delivery. You might experience slight bleeding, pain/discomfort at the site of the needle insertion. Very few may develop a bruise, discomfort, dizziness or infection. Cord blood collection will be done using a standard procedure and only if there are no risks for you or your baby.

For some people, hand expressing of breast milk can be difficult. The inconvenience you may experience is small. You will be given a pamphlet to explain how to hand express; also you can contact the nurse who gave you the kit for extra help. If you are having difficulty with the hand expression technique, you may use your breast pump to collect the requested amount of milk.

Some of the questions in the questionnaires are of a personal nature and may cause you some discomfort or anxiety. You can discuss this with the research nurse. You may choose not to answer any questions that make you feel uncomfortable.

The lab results for heavy metals and environmental chemicals may cause you some anxiety and we strongly recommend that you discuss them with your health care provider or local site investigator. There is also a small potential risk that a life or health insurance company may consider the lab results in your medical chart when determining your insurance premium.

7. Who will have access to my records and know that I am in a study?

Only the study team will have access to your records. Confidentiality will be respected and no information that contains your name or identity will be released or published without consent unless required by law. This legal obligation includes a number of circumstances, such as suspected child abuse, infectious disease, or expression of suicidal ideas where researchers are obliged to report to the appropriate authorities. As a partner or founder of this research, a member of Health Canada, the hospital research ethics committee, the Ontario Ministry of Environment and the Canadian Institutes of Health Research, will have the right to access the research data file and the medical chart for audit and quality control purposes only.

The lab results described above will be used for research purposes only to meet the purposes of this study. No names of individuals will appear on the questionnaires or on any of the samples collected, only a unique code. This unique code will be linked back to the information collected during the research study but only the local site investigator and study nurse team will have access to the key to link this code with your name and contact information. The consent forms and an electronic key file with your name and address will be kept separately in a secure location at the local study site.

Other coded information will be located in the study coordinating center at Ste. Justine's Hospital in Montreal, and temporarily at Génome Québec (for genome studies, these results will not be shared with you but will be kept confidential and will not be documented in your medical chart), at Institut National de Santé Publique du Québec (for analysis of environmental chemicals) and at Health Canada laboratories (for analysis of milk and hair as well as nutritional and other factors in blood and urine that might make you more or less likely to experience harmful effects of environmental chemicals).

We will notify your doctor that you are participating in this study if you have any results higher than health-based guidelines that are scientifically proven to be significant for your health and there are preventive measures or treatments.

The study samples will be kept until all chemical analyses are complete, and the study data will be kept until all MIREC related research papers have been published. The general results of this study will be presented during scientific conferences and published in scientific journals, but no information that would allow individuals to be identified will be presented.

8. Commercialization

The knowledge gained from your data and biological samples could contribute to the creation of commercial products or to the broader commercialization of existing products. However, you will not be entitled to share the potential economic benefits.

9. Who can participate in this study?

Pregnant women in each city selected for the study can participate if they are enrolled during weeks 6 – 13 of pregnancy, are 18 years or older, are able to consent, can speak and understand English or French and are generally healthy.

10. Who can I contact if I have further questions?

If you have further questions, you can call the local site investigator, Dr. William D. Fraser, at (514) 345-4931, extension 4948, or the research nurse, Susanne Andersen, extension 4334. For all information regarding your rights as a research project participant you can contact the CHU Sainte-Justine local quality service and complaints commissioner at (514) 345-4749.

11. Will I get paid for my participation in the study?

No payment will be provided for participating in this study.

12. Can I refuse to be in the study and can I be asked to leave the study?

Your participation in this study is strictly voluntary. You can choose not to take part in the study, or, if you choose to participate, you can quit at any time. Your decision not to participate or decision to withdraw from the study will in no way adversely affect the quality of care that you will receive during your pregnancy, or during and after delivery. In addition, you will not be prevented from participating in future studies. You may be asked to leave the study by the local site investigator without your consent if you do not follow the study plan, if you have a study-related injury or for any other reason. There are two levels of study withdrawals: you can withdraw partially from the study follow-up (e.g. study visits, questionnaires), but authorize the keeping of the data already collected as well as the data

collection from your clinical chart. You can also fully withdraw. In this case, we will destroy all your data and specimens collected. You will be asked to specify what level of withdrawal you choose.

13. Investigator's responsibility

In the unlikely event of problems resulting from a procedure during this study, you will receive all the care needed by your health status and covered by your hospitalization and medical insurance.

By signing this consent form, you are not, in any way, giving up your legal rights. Moreover you are not freeing the investigators or the sponsors from their legal and professional responsibilities.

14. Participant Follow-up

If funds become available at a later date, the researchers are interested in following you and your baby as he or she grows and develops to assess the health risks, if any, from exposure to these environmental chemicals during pregnancy. At this time, we are only asking if we can contact you again to see if you are interested in participating in these follow-up studies.
You will receive a signed copy of this consent form.

CONSENT:
By signing this form, I agree that:

Yes No Initials

- The MIREC study has been explained to me.
- All of my questions were answered.
- The possible risks discomforts and benefits of this study have been explained to me.
- I understand that I have the right not to participate and the right to withdraw at any time.
- I understand that I may refuse to participate without consequence to my continuing medical care.
- I am free now, and in the future to ask any questions about the study.
- I understand that my personal information will be kept confidential unless required by law to be disclosed
- I understand that no information that would identify me will be released or printed without asking me first.
- I understand that I will receive a signed copy of this consent form.
- I agree to be contacted after the birth of my infant to see if I am interested in further research on the health of my baby. ☐ ☐ _____
- The research staff may access my medical charts and those of my baby to record information on the health of my pregnancy and my baby.

In case I have moved during that time, you may contact this person, who should know my new address:

Name: _____

Address: _____

Phone number: _____

I was informed of the nature and the content of the research project. I have read the informed consent, and a copy has been given to me. I received answers to the questions that I asked. After thinking about it, I agree to participate with my child in this research project. I authorize the research team to access my and my child's medical records to collect the information pertinent to the project. I hereby consent to the participation of both myself and my child in this study.

_____	_____
Name of participant:	Age
_____	_____
Participant signature	Date

I consent that my spouse can answer questions related to my fertility, my work and my activities that could expose my family to environmental chemicals.

_____ _____

Name of the spouse of the participant Age

_____ _____

Spouse signature Date

I have explained to the participant all the significant aspects of the research and have answered the questions she asked me. I have explained that participation in this research project is free and voluntary and it can be stopped at any time.

_____ _____ _____

Name of person who obtained consent (block letters) Signature Date

The research project as well as the terms of participation must be described to the participant. A member of the research team must answer her questions and must explain her that participation in the research project is free and voluntary. The research team agrees to respect what has been agreed to in the consent form.

_____ _____ _____

Name of the responsible researcher Signature Date

Appendix 2: Informed consent for the MIREC study (collection of biological samples)

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Pour l'amour des enfants

Université de Montréal

INFORMED CONSENT

MIREC
Maternal-Infant Research on Environmental Chemicals

Maternal-Infant Research on Environmental Chemicals (MIREC): FUTURE RESEARCH ON STORED BIOLOGICAL SAMPLES (blood, urine, hair, breast milk, meconium)

Co- principal investigators:
Dr William D. Fraser — Obstetrician, CHU Ste-Justine
Tye Arbuckle — Epidemiologist, Health Canada

Local Site Investigator: Dr. William D. Fraser, M.D., M.Sc., FRCSC

1. Why is it important to store your biological samples for future research as part of the MIREC study?

Little is known about the potential long-term health effects, if any, of prenatal or early life exposure to the levels of environmental chemicals seen today. Studies have shown that some of these chemicals may affect the child's growth and development, including effects on behavior and ability to learn. However, this research remains preliminary and needs to be tested in larger populations such as the MIREC study. The environmental chemicals that will be measured in you and your baby were selected based on the current science and limited by the budget available. In future years, based on the latest scientific information (along with the funds to do the work), we would like to measure additional chemicals, as well as markers that might help us understand how a particular chemical causes harm and why some people are more likely to be exposed or suffer health effects. We may also want to do research on fetal growth, pregnancy and health of mothers and their babies. If we did not have your stored samples to test these new research questions, we would have to start a whole new study which would be very costly and result in long delays to obtain the results.

2. What is the purpose of this project?

The MIREC study is being conducted to fulfill three main purposes:
(1) To measure the extent to which pregnant women and their babies are exposed to environmental chemicals. Exposure will be measured in maternal blood, urine, hair, breast milk as well as cord blood and meconium (infant's first stools);
(2) To measure some of the beneficial components in human breast milk (i.e. nutrients and immune factors); and

(3) To assess what health risks, if any, are associated with the levels of heavy metals measured.

Another purpose of the study is to create a data and biological sample bank. Your coded data and samples (unused blood, urine, hair, cord blood, meconium and breastmilk) would be stored in this "bank" and used for future research on fetal growth, pregnancy, health of mothers and their children, and to measure new environmental chemicals.

3. Number of participants in this study
For the MIREC study, we aim to recruit approximately 2,000 women in their first trimester of pregnancy from 8 to 10 sites across Canada.

4. What will be the process of this study?
Future Research on the Data and Biological Sample Bank
To access the information and/or samples in the "bank", any future research would first have to be approved by the MIREC biological sample bank management committee to make sure that the research met the purposes of the MIREC study. The research plan would also have to be approved by the research ethics committees at Health Canada and Ste. Justine's Hospital (the coordinating center).

The type of data and samples that will be stored in the data and biological sample bank includes:

- Maternal blood, urine, breast milk and hair
- Newborn cord blood, and meconium
- Questionnaire and medical chart information from each visit:
    - Visit 1: between 6 and 13 weeks
    - Visit 2: between 16 and 21 weeks
    - Visit 3: between 32 and 34 weeks
    - Visit 4: Delivery
    - Visit 5: About 2 days after delivery
    - Visit 6: Home visit, between 2 to 8 weeks after delivery.

The study data will be stored in secure servers at CHU Sainte-Justine. All of the samples will be coded with a unique bar code so that they can be linked with the questionnaire and medical chart information collected. Only the local site investigator and study nurse will have the key to link the unique code to the names and contact information for the mothers and babies. The samples will be stored at CHU Ste-Justine's clinical research unit and at Health Canada in Ottawa in freezers at -20°C or -80°C (depending on what is required for proper storage of the sample). The data and any remaining biological samples will be destroyed after 30 years of storage, following strict procedures. If a participant wishes to withdraw from the study, she must contact her local site investigator or research nurse and request that her data and specimens be destroyed.

5. What advantages and benefits can I expect?
There is no direct benefit for you personally. The future research will be targeted at children's health and the environment. With new and emerging manufactured chemicals

being measured in the environment each year, these future studies may give new knowledge into the health risks, if any, that these chemicals may pose.

6. What are the possible risks for me or my baby?
There are no known risks to you and your baby. The biological samples and data will already have been collected for the MIREC study.

7. Who will have access to my stored records and samples for future research and know that I am in a study?
Researchers who will submit a proposal to the bank management committee will have access to your coded records and samples, if and only after their request has been approved by the bank management committee and the research ethics committees. Confidentiality will be respected and no information that discloses your name or identity will be released or published without consent unless required by law. This legal obligation includes a number of circumstances, such as suspected child abuse, infectious disease, or expression of suicidal ideas where research documents are ordered to be produced by a court of law and where researchers are obliged to report to the appropriate authorities. As a partner in this research, a member of Health Canada, the CHU Sainte-Justine and Health Canada ethics committees, the Ministry of Environment of Ontario and the Canadian Institutes of Health Research, will have the right to access the research data file and the medical chart for audit and quality control purposes only.

No names of individuals will appear on the questionnaires, in the data bank or on any of the samples collected, only a unique code. This unique code will be linked back to the information collected during the research study but only the local site investigator and his research team will have access to your name and contact information. The consent forms and an electronic file with your name and address will be kept separately in a secure location at the local study site.

8. Commercialization
The knowledge gained from your data and biological samples could contribute to the creation of commercial products or to the broader commercialization of existing products. However, you will not be entitled to share the potential economic benefits.

9. Who can participate in this study?
Anyone who has agreed to participate in the MIREC study can have their data and samples stored for future research on fetal growth, pregnancy, health of mothers and their babies and environmental chemicals.

10. Who can I contact if I have further questions?
If you have further questions, you can call the local site investigator; Dr. William D. Fraser at (514) 345-4931 extension 4948, or the research nurse, Susanne Andersen, at the extension 4334. For all information regarding your rights as a research project participant you can contact the CHU Ste-Justine local quality service and complaints commissioner at (514) 345-4749.

11. Will I get paid for my participation in the study?
No payment will be made for participating in the long-term storage of your data and samples for future research.

12. Can I refuse to have my data and samples stored for a maximum of 30 years for future research?
Your participation in the long-term storage of your data and samples for future research as part of the MIREC study is strictly voluntary. You can choose to participate in MIREC but not agree to the long-term storage of your data and samples for future research. You may also withdraw your consent for the long-term storage at any time by contacting the local research nurse or site investigator. Your decision not to participate or decision to withdraw from the study will in no way adversely affect the quality of care that you will receive during your pregnancy, or during and after delivery. In addition, you will not be prevented from participating in future studies.

13. Investigator's responsibility
By signing this consent form, you are not, in any way, giving up your legal rights. Moreover you are not freeing the investigators or the sponsors from their legal and professional responsibilities.

14. Results
Any biological samples used in future research will first require approval from the research ethics board.

Only group level results of any future research will be presented during scientific conferences and published in scientific journals. No information that would allow individuals to be identified will be presented.

You will receive a signed copy of this consent form.

CONSENT:

I understand that any future research that uses my stored data and samples will only be used if that research has been approved by the MIREC biological sample bank management committee and by the research ethics committees at Health Canada and Ste. Justine's Hospital.

I understand that I may withdraw my consent for the long-term storage of my data and samples for future research at any time by contacting the local site investigator or research nurse.

Mother's Specimens: I agree that my blood, urine, breast milk and hair may be stored and used for future research on fetal growth, pregnancy, and health of mothers and their children.

Baby's Specimens: I agree that my baby's cord blood and meconium may be used for future research on fetal growth, pregnancy, and health of mothers and their children.

_____	_____
Name of participant:	Age
_____	_____
Participant signature	Date

<u>Spouse of the participant:</u>
I consent to use my personal information collected in the questionnaires for the purpose of future research.

_____	_____
Name of the spouse of the participant	Age
_____	_____
Spouse signature	Date

I have explained to the participant all the significant aspects of the research and have answered the questions she asked me. I have explained that participation in this research project is free and voluntary and it can be stopped at any time.

_____	_____	_____
Name of person who obtained consent	Signature	Date

(block letters)

The research project as well as the terms of participation must be described to the participant. A member of the research team must answer her questions and must explain her that participation in the research project is free and voluntary. The research team agrees to respect what has been agreed to in the consent form.

_____ _____ _____

Name of the responsible researcher Signature Date

Appendix 3: Informed consent for the MIREC-ID study

CHU Sainte-Justine
Le centre hospitalier universitaire mère-enfant

Pour l'amour des enfants

Université de Montréal

INFORMED CONSENT

Maternal-Infant Research on Environmental Chemicals
- Infant Development -
(MIREC–ID)

Birth and 6-month visit

Investigators:

Team Leader: Gina Muckle, Ph.D. (Laval University)

Co- principal investigators: Tye Arbuckle Ph.D. (Health Canada), William D Fraser MD. (University of Montreal, Sainte-Justine Research Center), Gina Muckle, Ph.D. (Laval University), Jean R Séguin, Ph.D. (University of Montreal, Sainte-Justine Research Center), Bruce Lanphear MD. (Simon Fraser University, British Columbia Children's Hospital).

Co-investigators: Dave Sainte-Amour, Ph.D. (U of Montreal, Sainte-Justine Research Center), Éric Dewailly, MD. (Laval University), Patricia Monnier , MD. (University of Montreal Sainte-Justine Research Center), Benoit Jutras, Ph.D. (University of Montreal, Sainte-Justine Research Center), Christine Till, Ph.D. (York University), Michel Boivin, Ph.D. (Laval University), Ginette Dionne, Ph.D. (Laval University), Zhong Cheng Luo, MD (University of Montreal, Sainte-Justine Research Center), Shu Qin Wei, MD. (University of Montreal, Sainte-Justine Research Center), Warren G. Foster, Ph.D. (McMaster University), Linda Dodds, Ph.D. (Dalhousie University), Belkacem Abdous, Ph.D. (Laval University), Pierre Ayotte, Ph.D. (Laval University), Mark Walker, MD. (University of Ottawa, Ottawa Health Research Institute), Gideon Koren, MD (Hospital for Sick Children, Toronto).

Local Site Investigator: Dr. William D. Fraser, MD.

Funding agencies and research partners: Health Canada

1. Why is the MIREC-ID study being done?

We thank you for your participation in the Maternal-Infant Research on Environmental Chemicals (MIREC) study. The information that we have collected during your pregnancy will help us to determine what levels of various chemicals are found in the Canadian population and the effects, if any, of exposure to metals such as lead and mercury, and to persistent organic pollutants such as, polychlorinated biphenyls (PCBs), on pregnancy outcomes.

Now we are inviting you to participate in the MIREC-Infant Development study (MIREC-ID). These research activities will take place at birth and when your infant is 6 months of age. **The purpose of the MIREC-ID study is to examine whether there is a link between prenatal exposure to environmental chemicals, as collected in the MIREC study, and growth, sensory and sexual development, cardiac variability, and general development and behaviour during infancy**.

We know that not everyone who experiences the same risks will develop a certain health problem. In the MIREC study you agreed to let us test for characteristics that may make you and your baby more or less resistant to any harmful effects of environmental exposures (i.e. genomic testing to identify genes that could affect reactions to certain chemicals or diseases; nutritional status; socioeconomic characteristics). In MIREC-ID, we would like to determine if any nutritional, socioeconomic and psychosocial characteristics combined with environmental chemical exposure affects the risk of adverse infant health, developmental and behavioural outcomes.

Please read this consent form to decide whether you are interested in participating in this follow up study. Please take your time to make your decision, carefully read the following information and ask any questions you may have.

2. Number of participants in this study

We are inviting 1000 women across Canada who participated in the MIREC study to be part of this follow up. We expect to enrol between 100 and 200 infants *at CHU Sainte Justine*.

3. How will this study be conducted?

There are two phases where we will do some assessments of your baby: (A) within 24 to 48 hours of birth of your child before you leave the hospital and (B) when your baby reaches 6 months of age. The latter assessments will also be conducted at the hospital. Data obtained from your participation in the MIREC study will help us to determine in MIREC-ID, if exposure to environmental chemicals is associated with infant growth, development and behaviour.

A: At Birth

If you agree to participate in this new phase, a trained research nurse or a trained research assistant will conduct three additional procedures on your baby. These procedures are non -

invasive and involve limited discomfort for your baby and will take about 20 to 30 minutes. You will be present during these procedures. The research nurse or the research assistant will audio record his/her measurement results to ease the data recording procedures.

If your baby shows any signs of discomfort or cries during any of the procedures/measurements, the research nurse or research assistant will allow you to calm your baby, and try the procedure/measurements again with your permission. If at any time you are uncomfortable with the procedures/measurements, you may choose to stop the procedure or withdraw from that particular assessment.

1. **Neonatal blood spot:** (Newborn blood sample) As part of standard medical care, a blood sample is routinely taken from your baby by pricking your baby's heel or by using a needle into a vein of your baby's arm or hand before you leave the hospital. At the same time that this is being done, we will obtain 1 to 2 extra blood drops (around half teaspoon) on a filter paper, which will be stored in the MIREC Biobank and later used to measure markers that may be important in understanding the potential role, if any, of environmental chemicals on infant health. No additional needle prick will be done to obtain our blood sample.

2. **Growth measurements:** Your baby will be weighed and measured at birth including length, head and arm circumferences, and skin fold thickness.

3. **Genital exam:** This includes the review of your baby's medical chart and a general examination of the breast and genitals, including the position of testis and pigmentation (skin color) of the genitals, measurements of the penis size (if applicable) and anogenital distance (distance from the anus to the base of the penis or the clitoris).

   The color of the skin will be measured with a machine called a Mexameter. A probe attached to this machine will be applied gently onto the bottom of the back, the coloured skin area around the nipple, and the genital area of your baby to measure the skin color at these places.
   We will also take a cell sample from the vagina of girls for analysis. This sampling is conducted by a gentle application of a cotton swab onto the opening of the vagina for a period of 10 to15 seconds.

   These tests and measurements are indicators of potential effects of prenatal exposure to environmental chemicals on your baby's hormones (i.e. steroid hormones). Your baby may experience a little discomfort during these measures but they do not pose any risk to her/him. This is a relatively new area of research and as such we will not be able to interpret what any of these assessments mean to your baby's health.

B: At 6 months

The research nurse or the research assistant will call you to schedule a 2 to 4 hour visit at the hospital. At the beginning of this visit, we will place tiny sensors on your child's chest in

order to measure his or her heartbeat with a small portable instrument. Then, we will conduct a short eye examination and test the visual acuity (sharpness) of your infant by presenting cards. We will also do auditory (hearing) testing and examine brain activity while your child is looking at pictures on a computer monitor or listening to sounds. These tests will use special sensors placed on your child's head to record brain activity.

Following these assessments, your baby will be weighed and measured including length, crown-rump length, length of fingers, circumferences of the head and arm, and skin fold thickness. Additional assessments will consist of measuring the pigmentation (skin color) of the genitals of your baby. The research nurse or the research assistant may audio record his/her measurement results to ease the data recording procedures.

Lastly, to assess your baby's behaviour, your baby will be placed in a sitting position and the research nurse or the research assistant will gently hold your baby's forearms on each side of the trunk for 3 minutes while kneeling with her head down. Your baby will be able to see you if he/she turns their head. If your infant cries more than 20 seconds, we will end this procedure.

Your child's assessments at 6 months will be video recorded. This recording will be reviewed by our senior child tester to provide feedback to the research nurse or the research assistant on the quality of the assessments and for coding of the child's reaction during the arm restraint task.

You will also be interviewed about your background (e.g. education, occupation), lifestyles (e.g. smoking, alcohol consumption and drug use), prenatal stress, well-being, interaction with your partner and support you may receive from your family and neighbourhood. Other questions will be related to your baby's sleep, nutrition, health, childcare, general development and behaviour.

Finally, in a self-administered questionnaire you will be asked to answer some questions about how you feel, your relationship with your current partner, relationships between you and your baby, about your childhood, your adolescence and your adulthood. Some of the questions may be sensitive or upsetting for you. In that case, you may contact the study nurse or the study research assistant who will recommend appropriate resources to meet your needs. You will only be identified on this questionnaire by an identification code and all information will be kept confidential.

4. What advantages and benefits can I expect?

Your participation in this follow-up study may not directly benefit you or your baby. **The testing we will do as part of this study does not replace the regular medical follow-up of your baby by his or her own doctor.** However, your participation will help us to understand whether or not exposure to environmental chemicals is affecting the health of Canadian infants. If any health-related problems requiring medical consultation are found with your child during the 6 months visit, we will inform you so you can consult your baby's doctor. In case you do not have a medical doctor, we will refer you to the pediatrician of the research group, Dr Anne-Monique Nuyt.

5. What are the possible risks for me or my baby?

There are minimal risks to you and to your baby. For the additional blood drops, extra pricking should not be necessary. If an extra pricking is required, your baby's discomfort will last a few seconds more than what he/she may usually experience during the blood sampling procedure routinely done at birth. All other procedures are non-invasive and your infant should experience none to minimal discomfort. If your baby becomes irritable during any part of the exams we will stop the measurements to allow your baby to calm down and try the measurements again. If your baby remains upset we will stop the assessments. You may also ask us to stop the assessments at any time.

Some questions you will be asked are of a personal nature and may be of some discomfort to you, however, you can at any time refuse to answer any questions. The 6 month visit and questionnaire may take up to 2-4 hours and this may be an inconvenience to you. Should a situation of emotional, family or social difficulties arise and you do not have access to appropriate services, we will consult Dr. Martin St-André, psychiatrist of the team and we will recommend appropriate resources to meet your needs.

6. Who will have access to my records and know that I am in a study?

All information collected about you and your child during the course of this study will be kept confidential unless otherwise authorized by you or required by law. To achieve this end, you and your child will only be identified in these research records by an ID number. The research data, neonatal blood spots will be kept secured after the end of the study in the MIREC Data and Biobank for as long as Dr. William D. Fraser of the CHU Sainte-Justine Research Centre or someone appointed by him can guarantee their proper management, after which they will be destroyed.

Only the study team members at this site will have access to your personally identified records. However, for the purposes of auditing the proper conduct of the study and ensuring your protection, it is possible that a delegate from the CHU Sainte-Justine's Research Ethics Board and representatives from Health Canada will consult the research data file. Furthermore, the study's findings may be published or released at a scientific meeting, but no identifiable information about you or your child will be given at that time.

7. Who can I contact if I have further questions?

If you have further questions, you can call the local site investigator, *Dr. William D. Fraser at (514) 345-4931 extension 4948, or the research nurses, (Susanne Andersen, or Julie Savaria at 514-345-4931, extension 4334).* For all information regarding your rights as a research project participant you can contact hospital's Complaints Commissioner and the quality of services at 514- 345-4749.

8. Will I get paid for my participation in the study?

At the six month visit, you will be provided with $50 for expenses such as child care, parking fees and lunch and your child will receive a small gift.

9. Can I refuse to be in the study and can I be asked to leave the study?

Taking part in this study is voluntary. You can choose to take part in the study and later change your mind and withdraw from the study. In that case, if you request it, your data will be destroyed. You are also free to refuse to answer any questions or to stop any procedures or interview before it is over. Your decision will not change any present or future relatioships with your health care providers or any other services you and your child are entitled to receive.

Even though you volunteered to participate, to be eligible for this study, your infant must be a singleton without major congenital birth defects, or neurological disorders. In this situation, the investigator may decide not to include your baby in the study.

10. Investigator's responsibility

In the unlikely event of problems resulting from a procedure during this study, your baby will receive all the care and medications needed by your health status and covered by the provincial health insurance plan. By signing this consent form, you are not, in any way, giving up your legal rights. Moreover you are not freeing the investigators from their legal and professional responsibilities.

11. Participant Follow-up

If future funds become available, the researchers are interested in continuing to follow you and your baby as he or she continues to grow and develop. We would like to do this in order to assess the health risks, if any, from exposure to these environmental chemicals during pregnancy. At this time, we are only asking if we can contact you again to see if you are interested in participating in these follow-up studies.

You will receive a signed copy of this consent form.

CONSENT:

- I agree that the study has been explained to me, all my questions were answered to my satisfaction and the possible risks of the study have been described to me.
- I understand that I can refuse to participate, to withdraw at any time and to not answer specific questions without consequence to continuing medical care.
- I have read the informed consent, and a copy has been given to me. I agree to participate with my child in this research project.

	Yes	No	Initials
I agree to be contacted after this follow up to see if I am interested in further research on the health of my baby.	O	O	
I agree to the storage of my child's dried blood in the MIREC Biobank for future research.	O	O	

_____ _____

Name of participant parent Age

_____ _____

Participant signature Date

I have explained to the participant all the significant aspects of the research and have answered the questions she asked me. I have explained that participation in this research project is free and voluntary and it can be stopped at any time.

_____ _____ Name

of person who obtained consent (block letters) Signature

_____

Date

Appendix 4: Results of the univariate linear regression models

Results of the simple linear regression models (i.e. univariate linear regression) – Birth Cohort

	Anogenital Distance (male infants)	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	0.548 (-0.036, 1.133)	0.068
MBzP	0.271 (-0.306, 0.849	0.358
MCPP	-0.032 (-0.506, 0.443)	0.896
MEHHP	0.409 (-0.137, 0.955)	0.144
MEHP	0.438 (-0.180, 1.056)	0.166
MEOHP	0.535 (-0.043, 1.113	0.071
MEP	0.520 (0.057, 0.983)	0.029
TOTBPA	0.087 (-0.516, 0.690)	0.777
TOTTCS	0.075 (-0.237, 0.387)	0.639
TRICLOSANLIB	0.045 (-0.248, 0.338)	0.764
LMW_Pthalates[a]	0.800 (0.212, 1.387)	0.008
HMW_Phthalates[b]	0.711 (0.071, 1.350)	0.030
Specific Gravity	46.628 (-42.779, 136.036)	0.308
Infant birth weight	2.915 (1.580, 4.250)	0.000
Infant birth length	0.431 (0.141, 0.722)	0.004
Weight-for-length z-score	0.507 (-0.011, 1.024)	0.056

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate;

[a] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[b] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

	Anus-clitoris Distance (female infants)	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	-0.317 (-0.811, 0.178)	0.211
MBzP	-0.585 (-1.032, -0.138)	0.011
MCPP	-0.257 (-0.687, 0.173)	0.243
MEHHP	-0.164 (-0.675, 0.348)	0.531
MEHP	-0.138 (-0.720, 0.443)	0.642
MEOHP	-0.134 (-0.659, 0.391)	0.617
MEP	0.388 (0.012, 0.763)	0.045
TOTBPA	-0.318 (-0.834, 0.198)	0.229
TOTTCS	0.074 (-0.189, 0.336)	0.583
TRICLOSANLIB	-0.037 (-0.303, 0.230)	0.787
LMW_Pthalates[a]	0.800 (0.212, 1.387)	0.008
HMW_Phthalates[b]	0.711 (0.071, 1.350)	0.030
Specific Gravity	-8.733 (-91.991, 74.526)	0.837
Infant birth weight	1.822 (0.578, 3.066)	0.005
Infant birth length	0.057(-0.175, 0.290)	0.629
Weight-for-length z-score	0.829 (0.352, 1.306)	0.001

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate;

[a] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[b] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

	Anoscrotal distance (male infants)	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	-0.012 (-0.578, 0.555)	0.968
MBzP	0.067 (-0.488, 0.622)	0.814
MCPP	-0.083 (-0.539, 0.374)	0.723
MEHHP	0.215 (-0.308, 0.738)	0.422
MEHP	0.212(-0.375, 0.798)	0.480
MEOHP	0.224 (-0.331, 0.780)	0.430
MEP	-0.016 (-0.467, 0.435)	0.945
TOTBPA	0.330 (-0.229, 0.889)	0.248
TOTTCS	-0.026 (-0.318, 0.265)	0.859
TRICLOSANLIB	0.056 (-0.217, 0.330)	0.686
LMW_Pthalates[a]	0.358 (-0.157, 0.873)	0.174
HMW_Phthalates[b]	0.643 (0.089, 1.197)	0.023
Specific Gravity	25.430 (-60.550, 111.410)	0.563
Infant birth weight	2.505 (1.209, 3.801)	0.000
Infant birth length	0.442 (0.162, 0.721)	0.002
Weight-for-length z-score	0.288 (-0.214, 0.789	0.262

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate;

[a] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[b] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

	Anus-fourchette Distance (female infants)	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	0.014 (-0.018, 0.046)	0.398
MBzP	-0.223 (-0.693, 0.247)	0.355
MCPP	0.130 (-0.317, 0.577)	0.569
MEHHP	0.215 (-0.308, 0.738)	0.422
MEHP	0.378 (-0.217, 0.974	0.215
MEOHP	0.121 (-0.417, 0.660)	0.659
MEP	0.155 (-0.233, 0.543)	0.434
TOTBPA	0.173 (-0.385, 0.731)	0.545
TOTTCS	-0.040 (-0.326, 0.247)	0.787
TRICLOSANLIB	0.078 (-0.213, 0.368)	0.601
LMW_Pthalates[a]	0.358 (-0.157, 0.873)	0.174
HMW_Phthalates[b]	0.643 (0.089, 1.197)	0.023
Specific Gravity	2.246 (-3.100, 7.592)	0.411
Infant birth weight	0.048(-0.031, 0.128)	0.236
Infant birth length	0.002 (-0.013, 0.017)	0.821
Weight-for-length z-score	0.027(-0.005, 0.058)	0.099

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate;

[a] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[b] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

Results of the simple linear regression models (i.e. univariate linear regression) – 6-month Cohort

	2D:4D digit ratio (left hand, male infants)	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	0.000 (-0.005, 0.006)	0.869
MBzP	-0.001 (-0.007, 0.005)	0.716
MCPP	-0.002 (-0.006, 0.003)	0.496
MEHHP	-0.002 (-0.008, 0.003)	0.369
MEHP	-0.002 (-0.008, 0.003)	0.415
MEOHP	-0.003 (-0.009, 0.003)	0.303
MEP	0.001 (-0.003, 0.006)	0.582
TOTBPA	-0.002 (-0.007, 0.004)	0.594
TOTTCS	-0.002 (-0.005, 0.000)	0.098
TRICLOSANLIB	-0.003 (-0.006, -0.000)	0.037
LMW_Pthalates[a]	-.002 (-.010, 0.007)	0.727
HMW_Phthalates[b]	-.001 (-.011, 0.009)	0.863
Specific Gravity	0.185 (-0.712, 1.083)	0.686
Weight-for-length z-score	0.003 (-0.003, 0.001)	0.277

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate;

[a] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[b] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

	2D:4D digit ratio (left hand, female infants)	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	-0.003 (-0.009, 0.003)	0.323
MBzP	-0.003 (-0.009, 0.003	0.284
MCPP	-0.004 (-0.009, 0.001)	0.106
MEHHP	-0.006 (-0.012, 0.000)	0.085
MEHP	-0.006 (-0.013, 0.001)	0.105
MEOHP	-0.006 (-0.013, 0.001)	0.091
MEP	-0.003 (-0.007, 0.002)	0.260
TOTBPA	-0.004 (-0.011, 0.002)	0.172
TOTTCS	0.000 (-0.003, 0.004)	0.784
TRICLOSANLIB	-0.001 (-0.004, 0.002)	0.475
LMW_Pthalates[a]	-.001 (-.009, 0.008)	0.899
HMW_Phthalates[b]	0.000 (-.012, 0.013)	0.972
Specific Gravity	-1.070 (-2.090, -0.051)	0.041
Weight-for-length z-score	-0.005 (-0.013, 0.003)	0.251

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate;

[a] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[b] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

	2D:4D digit ratio (right hand, male infants)	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	0.001 (-0.005, 0.007)	0.780
MBzP	-0.001 (-0.006, 0.005)	0.857
MCPP	-0.004 (-0.008, 0.001)	0.131

MEHHP	-0.001 (-0.007, 0.004)	0.674
MEHP	-0.000 (-0.007, 0.006)	0.908
MEOHP	-0.001 (-0.007, 0.005)	0.764
MEP	0.000 (-0.004, 0.005)	0.863
TOTBPA	-0.007 (-0.013, -0.001)	0.024
TOTTCS	-0.000 (-0.003, 0.003)	0.882
TRICLOSANLIB	-0.000 (-0.004, 0.002)	0.627
LMW_Pthalates[a]	-.001 (-.009, 0.008)	0.862
HMW_Phthalates[b]	-.003 (-.013, 0.008)	0.634
Specific Gravity	-0.128 (-1.077, 0.822)	0.792
Weight-for-length z-score	-0.001 (-0.007, 0.006)	0.841

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate;

[a] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[b] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

	2D:4D digit ratio (right hand, female infants)	
Monoester metabolite	**Coefficient (95% CI)**	***P* value**
MnBP	-0.001 (-0.007, 0.006)	0.828
MBzP	0.002 (-0.004, 0.007)	0.579
MCPP	0.000 (-0.005, 0.006)	0.890
MEHHP	0.000 (-0.006, 0.007)	0.889
MEHP	0.004 (-0.004, 0.011)	0.336
MEOHP	0.001 (-0.006, 0.008)	0.810
MEP	0.003 (-0.002, 0.008)	0.218
TOTBPA	0.001 (-0.006, 0.007)	0.808
TOTTCS	0.002 (-0.002, 0.004)	0.404
TRICLOSANLIB	0.000 (-0.003, 0.003)	0.942
LMW_Pthalates[a]	0.004 (-.005, 0.013)	0.363
HMW_Phthalates[b]	0.002 (-.010, 0.015)	0.736
Specific Gravity	-0.046 (-1.096, 1.004)	0.931
Weight-for-length z-score	0.000 (-0.008, 0.009)	0.962

MnBP=Mono-n-butyl phthalate; MBzP=Mono-benzyl phthalate; MCPP=Mono-(3-carboxypropyl) phthalate; MEHHP=Mono-(2-ethyl-5-hydroxy-hexyl) phthalate; MEHP=Mono-(2-ethylhexyl) phthalate; MEOHP=Mono-(2-ethyl-5-oxo-hexyl) phthalate; MEP=Mono-ethyl phthalate;

[a] Low molecular weight phthalate monoester metabolites included: MMP, MEP, MnBP, MCHP. MCPP and MBzP.

[b] High molecular weight phthalate monoester metabolites included: MEHP, MnOP, MEOHP, MiNP, and MEHHP.